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(54) Title: DIAGNOSIS AND TREATMENT OF INFECTIONS DUE TO STREPTOCOCCI AND ENTEROCOCCI (57) Abstract <p>The present invention provides a purified bacterial protein expressed during infection due to streptococci or enterococci and isolated from human sera, together with immunogenic fragments, analogs, inhibitors, antibodies and antigenic fragments specific thereto. Also provided is a DNA sequence coding for a bacterial protein or an immunogenic fragment or an analogue thereof expressed during infection due to Streptococci or Enterococci, together with homologues thereof, together with vectors, probes and inhibitors therefor. Also provided is fibronectin or an immunogenic fragment thereof or an analogue thereof or an antibody thereto or an antigen binding fragment thereof when used in a method of treatment or diagnosis of the human or animal body for infection due to Streptococci or Enterococci. Also provided are antibodies specific to HSP 90 or immunogenic fragments or analogues thereof for use in a method of diagnosis or treatment of the human or animal body of infection due to streptococci or enterococci due to any one of the group of <i>S.oralis</i>, <i>S.gordonii</i>, <i>S.sanguis</i>.</p>		

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Diagnosis and Treatment of Infections
due to Streptococci and Enterococci

This invention concerns the diagnosis, prophylaxis and treatment of infections due to Streptococci and Enterococci, especially endocarditis and septicaemia.

Endocarditis is commonly caused by Streptococcal and Enterococcal infection. These are bacterial species which grow in the heart valves of an infected patient and cause damage thereto. Endocarditis is currently diagnosed by clinical features, echocardiogram and the presence of heart murmurs. The causative microorganism is usually identified by blood culture (culture-positive endocarditis). However, in approximately 10% of infective endocarditis patients the blood culture is negative. This may lead to a wrong diagnosis and/or delayed treatment.

Active infective endocarditis in which blood cultures are negative has been a recognised clinical entity since the beginning of the century. Etiological factors involved in such culture-negative endocarditis include (1) previous antibiotic therapy, (2) fastidious, slow growing bacteria and (3) non-bacterial organisms.

Patients with rheumatic fever, damaged heart valves or prosthetic valves are at risk of a secondary Streptococcal infection leading to endocarditis when having routine dental or gastrointestinal procedures.

Additionally, a growing problem in recent years has been the spread of

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vancomycin-resistant enterococci (VRE). The emergence of enterococci resistant to most or all licensed antibiotics leaves few treatment options and recent studies have shown that 36.6% of those patients with VRE in blood died as compared with 16.4% of those with vancomycin sensitive enterococci. Clearly a treatment for enterococci of all descriptions, and especially of VRE is extremely desirable.

One method of diagnosing endocarditis and, more particularly, culture-negative endocarditis, is by immunoblotting sera of such patients to reveal raised levels of antibodies against causative microorganisms and also a pattern on immunoblot which appears to be species specific (Clark & Burnie, J.Clin.Pathol. 1991, 44, 152-156; Burnie *et al.*, J. Clin. Pathol, 1987, 40: 1149-1158; Burnie & Clark, J. Immunol. Methods, 1989, 123: 217-225). However, although individual species of both *Streptococcus* and *Enterococcus* can be identified, there is some cross- reaction between species making absolute diagnosis sometimes difficult. Immunoblotting is also cumbersome as well as expensive.

Current therapy of both culture-positive and culture-negative endocarditis involves antibiotics, however, some of the antibiotics necessary to treat endocarditis are highly toxic, for example, vancomycin and gentamicin may be nephrotoxic and ototoxic. Additionally, it is difficult to assess patient response to antibiotic treatment since, although the organism may no longer be viable, fever may persist.

The present invention provides a method of diagnosis and treatment ("treatment" from herein being taken to include prophylaxis) of infections due to *Streptococci* and *Enterococci* which is specific to both culture-positive and culture-negative

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endocarditis, thereby overcoming at least to some extent, the aforesaid problems.

Experiments were undertaken which resulted in the cloning, sequencing and characterisation of Streptococcal antigens, resulting in the determination of an antigen expressed during infection due to Streptococci and Enterococci, together with immunogenic fragments thereof which were found to be useful in the prophylaxis and treatment of infections due to both Streptococci and Enterococci.

According to the present invention there is provided a purified bacterial protein expressed during infection due to streptococci or enterococci and isolated from human sera having at least the sequence of formula (1):

NH-	10	30	50
EFTFYDENDQ	PINFDNALLS	VASLNREHNS	IEMAKDYSGT FIKISGSSIG EKNGMIYATE
70	90	110	
TLNFKQGQGG	ARWTMYPNRQ	PGSGWDSSDA	PNSWYGAGAI SMSGPTNHVT VGATSATNVM
130	150	170	
SVAEMPQVPG	RDNTEGKRPN	IWYSLNGKIR	AVDVPKITKE KPTPPVAPTE PQAPTYEVEK
190	210	230	
PLEPAPVAPS	YENEPTPPVK	TPDQPEPSKP	EEPTYETEKP LEPAPVAPNY ENEPTPPVKT
250	270	290	
PDQPDPSKPE	EPNYETEKPL	EPAPVAPSYE	NEPTPPVKTP DQPEPSKPEE PNYDPLPTPP
310	330	350	
LAPTPKQLPT	PPAVPTVHFH	YNRLFAQPQI	NKEIKNEDGV DIDRTLVAQ SVVKFELKTE
370	390	410	
ALTAGRPKTT	SFVLVDPLPT	GYQFDLEATK	AASKGFETSY DKASHTVTFK ATEETLAAFN

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      430              450              470
ADLTksFETL YPTVVGRVLN DGATYTNnFT LTVNDATGVK SNIVRVTTpG KPNDPDNPNN
      490              510              530
NYIKPLKVNK NKQGVNIDGK EVLAGSTNYy ELTWDLdQYK GdKSSKEAIQ NGFYyVDDYP
      550              570              590
EEALTLQPEL VKIRDLEGNL VSGISVQQFD SLERAPKKVQ DLLKKANITV KGAFQLFSAD
      610
NPAEF

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or an immunogenic fragment thereof or an analogue thereof.

The single letters in formula (1) are each to be understood to represent a separate amino acid, and each is the conventional single letter symbol used for amino acids.

Bacterial proteins expressed during infection due to Streptococci or Enterococci according to the invention include those proteins wherein one or more amino acids in the sequence of formula (1) is replaced by another amino acid, providing that overall functionality of the protein is conserved.

A bacterial protein according to the invention may be further characterised by either one or both of the following features:-

- (1) It is an immunodominant conserved antigen; and
- (2) Recombinant human antibody in an animal model (mouse) protected against septicaemia infection;

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A bacterial protein according to the invention may also be characterised in that it is involved in binding to heart valves.

The bacterial protein may be obtained from one of the group of Streptococcus oralis, Streptococcus sobrinus, Streptococcus gordonii, Streptococcus sanguis, Streptococcus mutans, Streptococcus mitis, Streptococcus mitior, Streptococcus parasanguis, Streptococcus bovis, Enterococcus faecalis and Enterococcus faecium.

Additionally, the bacterial protein may be obtained from either one of the group of vancomycin-resistant Enterococcus faecalis and Enterococcus faecium.

Particular fragments of a bacterial protein expressed during infection due to Streptococci or Enterococci include any peptide epitopes ("immunogenic fragments"), for example, a few amino acids or analogues thereof. Examples of such epitopes include YEVEKPLEPAPVAPS, SYENEPTPPVKTPD, KTPDQPEPSKPEEPT, EPAPVAPSYENEPTP, YEVEKELVDLPVEPS, KTPDQNIPDKPVEPT, TMYPNRQPGSGWDSS and WYSLNGKIRAVDVPK. Peptides of this type may be synthesised using conventional liquid or solid phase peptide synthesis techniques.

In a further aspect the invention particularly provides a recombinant bacterial protein expressed during infection due to Streptococci or Enterococci having an amino acid sequence which includes at least the sequence of formula (1) or an immunogenic fragment thereof, or an analogue thereof.

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As mentioned earlier, current treatment of endocarditis is by antibiotics and recovery is often difficult to assess as fever may persist after other symptoms have been relieved. Tests currently available to measure the efficiency of antibiotic treatment, for example, minimum inhibitory concentration, minimum bactericidal concentrations and back titrations, measure only organism sensitivity not actual organism death in the patient. We show for the first time, as detailed below, that in patients with endocarditis due to Streptococcus oralis, S. gordonii, S. sanguis and S. mitis and undergoing antibiotic therapy, resolution of the disease was accompanied by at least a 50% drop in IgM titre within two weeks. This provides a direct marker of successful antibiotic therapy showing for the first time a direct marker of pathogen kill. Since the IgM antibody is specific to endocarditis it can be used in the diagnosis of both culture-positive and, more particularly, culture-negative endocarditis.

Thus the present invention also provides a method of diagnosis of culture-positive and culture-negative endocarditis using IgM antibody to a bacterial protein expressed during infection due to Streptococci or Enterococci, said bacterial protein, and therefore IgM antibody raised thereto, falling by 50% within two weeks after commencement of antibiotic treatment, thereby acting as a marker of pathogen kill.

In another use the bacterial protein according to the present invention may be employed, using conventional techniques, for screening to obtain activity inhibiting agents for use in the prophylaxis and treatment due to Streptococci or Enterococci and in particular of culture-positive and culture-negative endocarditis-causing bacterial infection. Such screening methods forms a further aspect of the invention.

In a further use, the bacterial protein according to the invention is particularly well suited for the generation of antibodies. Thus according to a further aspect of the invention we provide a bacterial protein expressed during infection due to Streptococci or Enterococci having an amino acid sequence which includes at least the sequence of formula (1) or an immunogenic fragment thereof or an analogue thereof, for use as an immunogen.

Standard immunological techniques may be employed with the bacterial protein in order to use it as an immunogen. Thus, for example, any suitable host may be injected with the protein and the serum collected to yield the desired polyclonal anti-bacterial protein antibody after purification and/or concentration. Prior to injection of the host the bacterial protein may be formulated in a suitable vehicle and thus according to a further aspect of the invention we provide a composition comprising a bacterial protein expressed during infection due to Streptococci or Enterococci and having an amino acid sequence which includes at least the sequence of formula (1) or an analogue thereof together with a pharmaceutically acceptable carrier, diluent or excipient.

For purification of any anti-bacterial protein antibody, use may be made of affinity chromatography employing an immobilised bacterial protein of the present invention as the affinity medium. Thus according to another aspect of the invention we provide a bacterial protein expressed during infection due to Streptococci or Enterococci having an amino acid sequence which includes at least the sequence of formula (1), or an immunogenic fragment thereof or an analogue thereof, covalently bound to an insoluble support.

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Various derivatives of the bacterial protein or fragment or analogue may be used to inhibit said protein, fragment or analogue. The use of the bacterial protein expressed during infection according to the invention as immunogens for the production of antibodies generates one type of inhibitor of the action of the protein. Generally, inhibitors of the bacterial protein are potentially useful in the diagnosis, and in particular the prevention and treatment, of infections due to Streptococci or Enterococci and in particular of both culture-positive and culture-negative endocarditis, and provide a further feature of the invention. Inhibitors include any antagonists of the action of the bacterial protein expressed during infection or agents which prevent their production, and in particular those which may be used in treatment of endocarditis-causing bacterial infections. Suitable inhibitors include, for example, pharmaceutical reagents, including antibodies, and chemical analogues of the bacterial protein expressed during infection to antagonise the action of the bacterial protein, and anti-sense RNA and DNA to prevent production of the bacterial protein. Suitable inhibitors may be determined using appropriate screens, for example, by measuring the ability of a potential inhibitor to antagonise the action of, or prevent the production of a bacterial protein expressed during infection due to Streptococci or Enterococci according to the invention or an immunogenic fragment thereof, or an analogue thereof, in a test model for example an animal model such as the mouse model.

It will also be appreciated that by suitable epitope mapping using conventional procedures[Geysen *et al.*, J. Immunol. Methods, 102: 259-274 (1987); Hopp and Woods, PNAS USA, 78(6): 3824-3828 (1981); Novotny *et al.*, PNAS USA, 83: 226-230 (1986)], peptide fragments of the bacterial protein expressed during infection may be identified which can be chemically synthesised. Synthetic peptide

antigens of this type may be used to produce inhibitors e.g. to raise antibodies for use in diagnosis and/or therapy, as previously described, or to produce antisera, e.g. non-specific polyclonal antisera, for use as a vaccine, and as discussed above form a further aspect of the invention.

According to a further aspect of the invention we provide a derivative of a bacterial protein expressed during infection due to Streptococci or Enterococci, said protein having an amino acid sequence which includes at least the sequence of formula (1) or an immunogenic fragment thereof or an analogue thereof, wherein the derivative inhibits said protein, fragment or analogue.

Such inhibitors may be used either alone or where appropriate in combination with other pharmaceutical agents, for example, other antibiotics.

One particularly useful group of inhibitors according to this aspect of the invention are antibodies capable of recognising and binding to the bacterial proteins.

Thus according to yet another aspect of the invention we provide isolated and purified antibody specific for one or more epitopes of a bacterial protein expressed during infection due to Streptococci or Enterococci having an amino acid sequence which includes at least the sequence of formula (1) or an immunogenic fragment thereof or an analogue thereof.

The antibody may be a whole antibody or an antigen binding fragment thereof and may in general belong to any immunoglobulin class. Thus, for example, it

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may be an immunoglobulin M antibody or, in particular, an immunoglobulin G antibody. The antibody or fragment may be of animal, for example, mammalian origin and may be for example of murine, rat or human origin. It may be a natural antibody or a fragment thereof, or, if desired, a recombinant antibody fragment, ie., an antibody or antibody fragment which has been produced using recombinant DNA techniques.

Particular recombinant antibodies or antibody fragments include human recombinant antibodies and in particular include (1) those having an antigen binding site at least part of which is derived from a different antibody, for example those in which the hypervariable or complementarity determining regions of one antibody have been grafted into the variable framework regions of a second, different antibody (as described in European Patent Specification No 239400); (2) recombinant antibodies or fragments wherein non-Fv sequences have been substituted by non-Fv sequences from other, different antibodies (as described in European Patent Specification Nos 171469, 173494 and 194276); or (3) recombinant antibodies or fragments possessing substantially the structure of a natural immunoglobulin but wherein the hinge region has a different number of cysteine residues from that found in the natural immunoglobulin but wherein one or more cysteine residues in a surface pocket of the recombinant antibody or fragment is in the place of another amino acid residue present in the natural immunoglobulin (as described in International Patent Application Nos PCT/GB88/00730 and PCT/GB88/00729).

The antibody or antibody fragment may be of polyclonal, or preferably, monoclonal origin. It may be specific for a single epitope or for a number of epitopes associated with the bacterial protein.

Antigen binding antibody fragments include, for example, fragments derived by proteolytic cleavage of a whole antibody, such as F(ab')₂, Fab' or Fab fragments, or fragments obtained by recombinant DNA techniques, for example Fv fragments (as described in International Patent Application No PCT/GB88/0747).

The antibodies according to the invention may be prepared using well-known immunological techniques employing the bacterial protein expressed during infection as antigen. Thus, for example, any suitable host may be injected with the bacterial protein and the serum collected to yield the desired polyclonal antibody after appropriate purification and/or concentration (for example by affinity chromatography using the immobilised bacterial protein as the affinity medium). Alternatively, splenocytes or lymphocytes may be recovered from the bacterial protein-injected host and immortalised using for example the method of Kohler *et al.*, Eur. J. Immunol. 6: 511, 1976, the resulting cells being segregated to obtain a single genetic line producing monoclonal anti-streptococcal or -enterococcal bacterial protein antibodies. Antibody fragments may be produced using conventional techniques, for example, by enzymatic digestion with pepsin or papain. Where it is desired to produce recombinant antibodies according to the invention these may be produced using for example the methods described in European Patent Specification Nos 171469, 173494, 194276 and 239400.

Antibodies according to the invention may be labelled with a detectable label or may be conjugated with effector molecule for example a drug eg. an antibacterial agent or a toxin or an enzyme, using conventional procedures and the invention extends to such labelled antibodies or antibody conjugates.

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The antibodies according to the invention have a diagnostic and/or preventative and/or therapeutic use. Thus for diagnostic use the antibodies may be employed to detect whether the bacterial protein is present in a host organism, to confirm whether the host has a particular Streptococcal or Enterococcal bacterial infection, and especially to test for the presence of such organisms in culture-negative endocarditis, and/or to monitor the progress of therapeutic treatment of such infections. Diagnostic methods of this type form a further aspect of the invention and may generally employ standard techniques, for example, immunological methods such as enzyme-linked immunosorbent methods, radioimmuno methods, latex agglutination methods or immunoblotting methods.

Antibodies according to the invention also have a therapeutic use in the treatment of bacterial infection due to Streptococci or Enterococci, for example, those just described and may be used alone or conjugated to an effector molecule, in the latter case to target the effector molecule, eg an antibacterial agent, to the infecting organism. For therapeutic use the antibody may be formulated in accordance with conventional procedures, for example, with a pharmaceutically acceptable carrier or excipient, eg., isotonic saline for the administration at an appropriate dosage, depending on the nature of the infection to be treated and the age of the patient.

If desired, mixtures of antibodies may be used for diagnosis and/or prevention and/or treatment, for example mixtures of two or more antibodies recognising different epitopes of a bacterial protein according to the invention, and/or mixtures of antibodies of a different class, eg., mixtures of IgG and IgM antibodies recognising the same or different epitope(s) of a bacterial protein of the invention.

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The protein or any fragment, analogue, inhibitor, antibody or antigen binding fragment thereof according to the invention may be used in a method of treatment or diagnosis of the human or animal body.

The diagnostic test method may be selected from one of the group of enzyme-linked immunosorbent assay, radioimmunoassay, latex agglutination assay and immunoblot assay.

Such a protein, fragment, analogue, inhibitor, antibody or antigen binding fragment may form part of a composition for use in a method of diagnosis or treatment of the human or animal body together with a pharmaceutically acceptable carrier, diluent or excipient.

The bacterial proteins according to the invention may be prepared by a variety of processes, for example, by protein fractionation from appropriate bacterial cell extracts, using conventional separation techniques such as ion exchange and gel chromatography and electrophoresis, or by the use of recombinant DNA techniques, as more particularly described in the "Experiments" section hereinafter. The use of recombinant DNA techniques is particularly suitable for preparing substantially pure bacterial proteins according to the invention.

Thus according to a further aspect of the invention we provide a process for the production of a bacterial protein expressed during infection due to Streptococci or Enterococci having an amino acid sequence which includes at least the sequence of

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formula (1) or a fragment or an analogue thereof, comprising the steps of (1) culturing a host organism transformed with a vector including a gene coding for a precursor of said protein and (2) recovering said protein.

Preferably the precursor cleaved in this aspect of the invention is a fusion protein comprising at least a portion of a protein produced in a transformed host organism and at least the amino acid sequence of formula (1). Such fusion proteins form a further aspect of the invention. Desirably the fusion protein includes a protein produced at a high level by a transformed host organism. Suitable such proteins include at least a portion of a chloramphenicol acetyltransferase (CAT) protein or, preferably at least a portion of the B-galactosidase protein.

According to a still further aspect of the invention we provide a DNA sequence coding for a bacterial protein or an immunogenic fragment or an analogue thereof expressed during infection due to Streptococci or Enterococci having substantially the nucleotide sequence of formula (2):

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5'      10              30              50
GAATTCACCT TCTACGATGA AAATGACCAA CCAATTAATT TTGACAATGC TCTTCTTTCA
      70              90              110
GTAGCCTCAC TTAACCGTGA GCATAACTCT ATTGAGATGG CTAAGGATTA TAGTGGTACT
      130             150             170
TTTATTAAAA TCTCAGGTTC ATCCATCGGT GAAAAAATG GCATGATTTA TGCCACAGAA
      190             210             230
ACCCTGAACT TTAACAAGG ACAGGGTGGG GCTCGCTGGA CAATGTATCC AAATCGTCAG

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250	270	290
CCAGGTTTCAG	GTTGGGATTC	ATCAGATGCA
CCAAACTCTT	GGTACGGTGC	AGGGGCCATT
310	330	350
AGTATGTCCG	GTCCTACGAA	TCACGTTACA
GTTGGTGCAA	CATCTGCTAC	CAATGTGATG
370	390	410
TCCGTAGCAG	AAATGCCTCA	AGTACCTGGA
AGAGACAATA	CTGAAGGTAA	AAGACCAAAC
430	450	470
ATCTGGTACT	CACTCAATGG	TAAAATTCGT
GCGGTTGACG	TTCCGAAAAT	TACAAAAGAA
490	510	530
AAACCAACTC	CACCGGTAGC	ACCAACTGAA
CCACAAGCTC	CTACCTATGA	AGTGGAGAAA
550	570	590
CCACTGGAAC	CGGCTCCAGT	AGCACCAAGC
TACGAAAATG	AGCCAACTCC	ACCAGTAAAA
610	630	650
ACTCCAGATC	AACCGGAGCC	ATCAAAACCA
GAAGAGCCAA	CATATGAGAC	AGAGAAACCA
670	690	710
TTGGAACCAG	CTCCAGTAGC	ACCAAACCTAC
GAAAATGAGC	CAACTCCACC	AGTAAAAACT
730	750	770
CCAGATCAAC	CAGACCCATC	AAAACCGGAA
GAGCCAAACT	ATGAGACAGA	GAAACCATTG
790	810	830
GAACCAGCTC	CAGTAGCACC	AAGCTATGAA
AATGAGCCAA	CTCCACCGGT	AAAAACTCCA
850	870	890
GATCAACCAG	AGCCATCAAA	ACCAGAAGAG
CCAAATTATG	ATCCATTGCC	AACTCCGCCG
910	930	950
CTAGCACCAA	CTCCTAAGCA	GTTGCCAACA
CCACCAGCGG	TGCCAACAGT	TCACTTCCAT
970	990	1010
TACAATCGTC	TATTTGCACA	ACCTCAGATT
AATAAAGAAA	TTAAAAACGA	GGATGGAGTA
1030	1050	1070
GATATTGATC	GTACTCTAGT	TGCTAAGCAG
TCTGTAGTGA	AGTTTGAGCT	GAAAACAGAA
1090	1110	1130
GCTTTAACTG	CTGGTCGTCC	AAAAACAAC
TCGTTTGTAT	TGGTAGATCC	ACTTCCAAC

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1150	1170	1190
GGCTATCAGT	TTGATTTGGA	AGCAACCAAG
1210	1230	1250
GACAAAGCTA	GTCACACTGT	AACCTTTAAG
1270	1290	1310
GCTGATTTGA	CAAAATCCTT	TGAGACTCTA
1330	1350	1370
GATGGGGCGA	CTTATACGAA	TAACCTTTACA
1390	1410	1430
TCAAACATTG	TTCGTGTAAC	GACTCCAGGT
1450	1470	1490
AACTACATCA	AGCCTTTGAA	AGTTAACAAG
1510	1530	1550
GAAGTTCTAG	CTGGTTCAAC	GAAGTTCTAG
1570	1590	1610
GGAGATAAAT	CTTCTAAAGA	AGCGATTCAA
1630	1650	1670
GAAGAAGCTT	TAACGCTTCA	ACCAGAATTG
1690	1710	1730
GTATCAGGTA	TCAGTGTTCA	ACAGTTTGAT
1750	1770	1790
GATCTGTTGA	AGAAAGCAAA	CATCACTGTT
1810		
AATCCAGCTG	AATTC	

and homologues thereof.

A DNA sequence according to the invention may be further characterised in that the bacterial protein for which it encodes may be characterised by either one or

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both of the following features:-

- (1) It is an immunodominant conserved antigen; and
- (2) Recombinant human antibody in an animal model (mouse) protected against septicaemia infection;

A DNA sequence may also be further characterised in that the protein for which it encodes is involved in binding to heart valves.

DNA with this sequence may be obtained from bacterial genomic DNA as described in the "Experiments" section hereinafter.

The DNA sequence according to this aspect of the invention may be incorporated in an expression vector using conventional techniques. Thus in a further aspect of the invention we provide an expression vector including substantially a DNA sequence of formula (2) or a homologue thereof.

The vector may be adapted for use in a given host cell by the provision of suitable selectable markers, promoters and other control regions as appropriate. Host cells transformed with such vectors form a further aspect of the invention. Suitable host organisms include bacteria (eg. *E.coli*), and mammalian cells in tissue culture.

The DNA sequence of formula (2) may also be used to design DNA probes for use in identifying the presence of Streptococcal and Enterococcal bacteria in the infected state and the invention extends to such DNA probes. Such probes may also be of use for detecting circulating bacterial nucleic acids, for example using a polymerase

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chain reaction, as a method of diagnosing such bacterial infections. The probe may also be synthesised using conventional techniques and may be immobilised on a solid phase, or may be labelled with a detectable label.

A DNA sequence, vector, probe or inhibitor according to the invention may be used in a method of treatment or diagnosis of the human or animal body.

It is known that Streptococci and Enterococci cause endocarditis by binding to heart valves and causing damage thereto. A possible mode of action of Streptococci in binding to heart valve tissue, or prosthetic valves, has been proposed to involve pre-binding of Streptococci to extracellular matrix proteins, such as fibronectin, which leads to the adherence and colonisation of bacteria to damaged valvular surfaces (Lowrance *et al.*, J. Clin. Invest., 1990, 86: 7-13). This suggests that fibronectin acts as a tissue receptor for the bacteria. However, we show for the first time that the prior art theory on the role of fibronectin in endocarditis is incorrect. In our studies, described below, antibody against fibronectin binds to PAc and is neutralised by prior crossabsorption with fibronectin indicating that PAc acts like fibronectin binding to damaged heart valves directly, and not via fibronectin. This molecular mimicry is the probable mode of action of PAc.

Thus the invention also provides fibronectin or an immunogenic fragment thereof or an analogue thereof or an antibody thereto or an antigen binding fragment thereof for use in a method of treatment or diagnosis of the human or animal body for infection due to Streptococci or Enterococci.

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Additionally, as described below in the "Experiments" section, we have shown for the first time that serum from patients with endocarditis due to S.oralis, S.gordonii and S.sanguis infection have antigen of approximately 85 kDa which reacts with a mouse monoclonal antibody specific to heat shock 90 molecules. We have also shown that in a mouse *S. oralis* infection model with death as an end point, antibody specific to HSP90 shows a statistically significant increase in survival. This shows that this antigen is in the HSP 90 group. Thus antibodies against HSP 90 may be used for the diagnosis and treatment of infections caused by S.oralis, S.gordonii and S.sanguis.

Thus the present invention provides antibodies specific to HSP 90 or immunogenic fragments or analogues thereof for use in a method of diagnosis or treatment of the human or animal body of infection due to streptococci or enterococci due to any one of the group of S.oralis, S.gordonii and S.sanguis.

The invention will be further apparant from the following "Experiments" section which exemplifies the invention.

Experiments

1. Characterisation of the Antibody Response

Sera was available from the following cases:

- 12 cases of septicaemia due to Streptococcus oralis,
- 14 cases of endocarditis due to Streptococcus gordonii,
- 2 cases of endocarditis due to Streptococcus oralis,
- 2 cases of endocarditis due to Streptococcus sanguis,
- 20 control sera from patients having no clinical evidence of endocarditis who were non-neutropenic, and
- 20 control sera from patients having no clinical evidence of endocarditis who were neutropenic.

2. Species Identification

Using the above sera, the causative organisms were identified, when available, according to the scheme of Beighton *et al.*, J. Med. Microbiol., 1991, 35: 367-372. Specifically, isolates from 6 of the cases of S.oralis were available and identified as S.oralis by JM Hardie (personal communication). The others were aesculin-negative and raffinose-negative -viridans streptococci eliminating S.gordinii (100% aesculin positive), S.mitis (100% raffinose positive) and making it unlikely to be S.sanguis (75% aesculin positive and 75% raffinose positive). In the 14 cases of S.gordonii and 2 cases of S.sanguis endocarditis, isolates from 3 were positively identified as S.gordonii. All the other isolates were unobtainable but the original API

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Streptococcal profile showed they were aesculin positive. This would eliminate S.mitis and make it unlikely that they were S.oralis (18% aesculin positive). They were then subdivided into either S.gordonii or S.sanguis according to whether the more dominant IgM response on immunoblot was against S.gordonii or S.sanguis. The cases of S.oralis endocarditis were identified according to Beighton *et al.* 1991.

3. Source of Strains for Immunoblotting

Using the protocol outlined by Burnie *et al.*, J. Clin. Pathol., 1987, 40: 1149-1158, antigenic extracts were made from S.sanguis NCTC 7863, S.oralis NCTC 7864 and S.gordonii NCTC 7868.

4. Immunoblotting

Immunoblotting using the above strains was then carried out as described in Burnie *et al.*, 1987.

The results of immunoblots of the patients sera described above is summarised in Table 1 (S.oralis), Table 2 (S.gordonii) and Table 3 (S.sanguis) and in Figures 1 to 5.

Figure 1 shows the immunoblot for S.oralis septicaemias. Paired IgMs and paired IgGs covering pre and post sera from 3 of the cases showing the antibody changes.

Figure 2 shows the immunoblot of S.gordonii endocarditis. Anti-fibronectin antibody

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against S.gordonii NCTC 7868 in Track 1, crossabsorbed against fibronectin in Track 2. Tracks 3-8 show IgM and IgG respectively in 3 cases of S.gordonii endocarditis.

Figure 3 shows 5 further case of S.gordonii endocarditis versus S.gordonii antigen Tracks 1/2, 3/4, 5/6, 9/10 and 11/12. Tracks 7/8 show pre-treatment of antibody levels of the same case as tracks 9/10 showing increased IgM to bands at approximately 85 KDa and IgG to bands at approximately 180, 58, 56 and 52 Kda.

Figure 4 shows the immunoblot of S.oralis endocarditis, Tracks 3/4 IgM and IgG, antifibronectin antibody crossreacting with 180 KDa band of S.oralis, Track 1 and effect of crossabsorption with fibronectin, Track 2.

Figure 5 shows the immunoblot of S.sanguis endocarditis, showing both IgM (track 3 and 5) and IgG (tracks 4 and 6) respectively of both cases, antifibronectin antibody crossreacting with the 120 KDa band of S.sanguis, Track 1 and the effect of crossabsorption with fibronectin, Track 2.

Comments

(a) S.oralis septaceamia

IgM and/or IgG against band at approximately 180 KDa when recovering from infection.

(b) S.gordonii endocarditis

Additional IgG was detected against bands at approximately 185 (1 case), 165 (2 cases), 155 (1 case), 140 (1 case), 132 (1 case), 110 (1 case), 94 (1 case), 61 (1

case), 50 (1 case) and 45 KDa (1 case). Additional IgM was detected against the 45 KDa band (1 case). The majority of cases has IgM and all IgG against the bands at 85 and 180 KDa from S.gordonii NCTC 7868. Other immunodominant bands included those at approximately 65 and 47 KDa.

(c) S.oralis endocarditis

case 1: Had additional antibody as follows. IgM against bands at approximately 180, 140 and 65 KDa and IgG against bands at approximately 180, 140, 120, 58, 51, 46 and 35 KDa. Both cases had IgM and IgG against the bands at 85 and 180 KDa from S.oralis NCTC 7864.

(d) S.sanguis endocarditis

Both patients produced antibody (IgM and IgG) against bands at approximately 120 and 85 KDa from S.sanguis NCTC 7863.

(e) S.sobrinus/S.mutans endocarditis

Immunoblotting demonstrated that patients with endocarditis had antibody (IgM and IgG) against three bands at approximately 185, 200 and 220 KDa.

Additionally the bands at 85 KDa (S.oralis NCTC 7864, S.gordonii NCTC 7868 and S.sanguis NCTC 7863) all react with a mouse monoclonal specific to heat shock 90 molecules (versus LKVIRK, see our previous patent application Nos WO 92/01717, WO 91/00351 and GB 2270076) showing that this molecule is in the HSP 90 group (Figure 6), Track 1 S.oralis NCTC 7864, Track 2 S.gordonii NCTC 7868, Track 3 S.sanguis NCTC 7863, Track 4 clinical isolate of S.oralis.

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5. Cloning of the immunodominant antigen of *S.sobrinus* and *S.oralis*

5.1 *S.sobrinus* cloning

(i) DNA isolation and lambda ZAPII library preparation.

Strain *S.sobrinus* Manchester University Collection of Bacteria No.263 (MUCOB 263), was used as the source of *S.sobrinus*. Initially, the MUCOB 263 organism was identified as an *S.mutans*, a fact reflected in the aboved mentioned publication. However, a more detailed testing of theis strain revealed that MUCOB 263 is biochemically more similar to *S.sobrinus* (Professor D. Bratthall, personal communication). To avoid future confusion, the organism has now been renamed *S.sobrinus* MUCOB 263. Bacterial cells were grown overnight at 37°C, with shaking, in brain-heart infusion broth containing 0.2% glucose and 40mM D,L-threonine (Sigma). The bacteria were harvested by spinning at 5,000 rpm for 10 mins before being washed, and resuspended in 12.5ml of 0.02 M Tris pH 8.2. Then 25 ml of 20 M polyethylene glycol (PEG) 24% in distilled water was added and mixed. Lysozyme was added at 34.6 mg/ml equivalent to 100µg/unit obtained by measuring the OD600 of a tenfold dilution of bacterial cultures. After 1 hour at 37°C, the spheroblasts formed were spun down at 5,000rpm for 10 mins and thoroughly resuspended in 50 ml of 10 mM Tris-Cl pH 8.0, 1 mM EDTA (TE). The spheroblasts were lysed with 5.5 ml of 10% sodium dodecyl sulphate (SDS) at 60°C for 15 mins. The DNA was purified by standard procedures (Maniatis *et al.*, Molecular Cloning - A laboratory manual, 2nd edn. Cold Spring Harbour, New York, 1989), RNAase A (sigma) treated (after preboiling the RNAase at 100°C for 15 mins), proteinase K treated, phenol:chloroform:isoamylalcohol (25:24:1) extracted, and ethanol precipitated. Plasmid DNA was removed by ethanol precipitation in the presence of 0.3 M ammonium acetate pH 5.2. Further purification was obtained

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by dialysis against TE over 36 hrs at 4°C.

The DNA was mechanically sheared, EcoRI linkers added, fractionated and inserts ligated into lambda ZAPII vector arms. The library had an insert size range of 2kb-7kb.

(ii) Antibody Screening

Serum was taken from a patient with endocarditis and used for antibody screening. Escherichia coli XL1 - Blue cells were infected at approximately 3,000 pfu/85mm on L broth agar (Bacto-tryptone 10 g/l, yeast extract 5 g/l, sodium chloride 10 g/l, maltose 2 g/l, bacto-agar 15 g/l). Plaques were transferred to nitrocellulose filters (0.45 µm pore size, Sartorius AG, Gottingen, Germany), impregnated with 10 mM isopropyl B-D-thiogalactopyranoside (IPTG), at 37°C for 2 hrs, after a 42°C incubation for 3 hrs. These filters were blocked overnight at 4°C with 3% bovine serum albumin (BSA - Sigma) in buffered saline (150 mM NaCl, 10 mM Tris). Patient serum, diluted one hundredfold in 3% BSA, was added to the filters and incubated at room temperature for 2 hrs, the filters then being washed for 30 mins in washing solution (150 mM NaCl, Tween 20, 0.05%), before the second antibody, a thousandfold dilution of anti-human IgG alkaline phosphatase conjugate (Sigma) in BSA 3% was added. After 1 hr at room temperature, the filters were again washed and stained with equal volumes of naphthol ASMX phosphate (Sigma, 0.4 mg/ml in distilled water) and Fast Red TR salt (Sigma, 6 mg/ml in 0.2 M Tris pH 8.2) (the Fast Red stain). Positive plaques were transformed to 1.5 ml tubes containing 200 µl of SM (100 mM sodium chloride, 50 mM Tris-Cl pH 7.5, 10 mM magnesium sulphate, gelatine, 0.0001%), and 2 to 3 drops of chloroform. Plaque

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purification was performed by the above method.

(iii) Antigen-directed antibody selection

Two 100 μ l aliquots of resuspended E.coli XL1-Blue cells were infected with a dilution of a high titre stock of purified positive phage, to give 5,000 pfu / 85 mm L broth agar plate. Phage adsorption occurred at 37°C for 30 mins. The adsorption mixes were added to 2.5 ml aliquots of L broth agarose 0.8%, mixed, and poured onto L broth agar plates. These plates were incubated at 42°C for 2.5 hrs, before nitrocellulose filters (0.45 μ m pore size, Sartorius), presoaked in 10 mM IPTG and dried at room temperature for at least 1 hr, were added and incubated at 37°C overnight. Each filter was then washed 3 times over 30 mins in Tris-buffered saline (150 mM sodium chloride, 10mM Tris-Cl pH 7.2), and blocked overnight at 4°C in 3% BSA. A tenfold dilution of patient serum in BSA 3% was added to the filters and incubated, with shaking, at room temperature for 3 hrs. This serum was removed, called the depleted serum and stored at 4°C. The filters were washed 5 times over 100 mins with Tween 20-Tris-buffered saline (150 mM sodium chloride, 10 mM Tris-Cl pH 7.2, Tween 20, 0.05%), and once with salt - Tween 20 solution (150 mM sodium chloride, Tween 20, 0.05%). The bound antibody was eluted by adding 5 ml of glycine saline buffer (150 mM sodium chloride, 200 mM glycine-Cl pH 2.8), and shaking at room temperature for 30 mins. The buffer was aspirated into a bijoux, containing Tris 0.04g per 100 μ l, mixed, termed the eluted antibody and stored at 4°C. A Western blot of S.sobrinus MUCOB 263 was performed essentially by the immunoblot method above with the original serum, depleted serum and eluted antibody being used as the primary antibodies. The antigen was the supernatant of the strain following crushing in an X-press. The secondary antibody was a

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thousandfold dilution of anti-human IgM and IgG alkaline phosphatase conjugates in 3% BSA, and the stain was the alkaline phosphatase stain.

Results

Six antibody positive clones were isolated and purified from the 60,000 pfu initially screened with culture-positive endocarditis patient serum. Antigen-directed antibody selection showed that the cloned, expressed sequence contained epitopes shared by S.sobrinus as the eluted antibody bound to the approximately 185 KDa antigen.

(iv) In vivo excision of DNA insert

A 10 ml overnight culture of E.coli XL1-Blue cells grown at 37°C in L broth, containing tetracycline 125 µg/µl, was spun down at 2,000 rpm for 10 mins, resuspended in 4 ml of 10 mM magnesium sulphate, and stored at 4°C. In a 50 ml plastic conical tube, 200µl of the resuspended E.coli XL1-Blue cells, 180µl of high titre antibody-positive phage (1.78 x 10⁸/85 mm plate approx) and 1 µl of R408 helper phage were combined and incubated at 37°C for 15 mins. (Helper phage and E.coli XL1-Blue cells from CLONTECH). Then, 5 ml of 2 x YT media (sodium chloride 5 g/l, yeast extract 10 g/l, bacto-tryptone 16 g/l pH 7.0) was added to the dual-infected bacteria and incubated, with shaking, at 37°C for 3 hrs. The cells were killed by heating at 70°C for 20 mins and ruptured by vortexing for 5 mins. The cells were spun at 4,000 rpm for 5 mins, and the supernatant containing pBluescript SK(-) phagemid decanted and stored at 4°C. The phagemid was propagated by adding 10 µl of the phagemid solution to 200 µl of resuspended E.coli XL1-Blue cells, incubating the culture at 37°C for 15 mins, and

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plating 1 μ l, 25 μ l, 75 μ l and 100 μ l aliquots onto L broth agar plates, containing ampicillin 50 μ g/ml.

(v) Plasmid DNA preparation and denaturation

The pBluescript SK(-) plasmid DNA was purified from the bacterial colonies with the Magic (RTM) Minipreps DNA purification System (Promega) and alkaline denatured by the method of Maniatis *et al.*, 1989. Hence, 8 μ l of solution, containing DNA 1.5 - 200 μ g was added to 2 μ l of 2 M sodium hydroxide, briefly vortexed and spun, and left at room temperature for 10 mins. Then 3 μ l of 3 M sodium acetate (pH 4.8), 7 μ l of distilled water, and 60 μ l of -20°C absolute ethanol were added and the DNA precipitated at -70°C for 30 mins. The precipitate was pelleted at 13,000 rpm for 1 min, washed with -20°C ethanol 70%, and vacuum-dried. The dried pellets were stored at -20°C.

(vi) DNA sequencing

DNA sequencing was performed by the two-step chain-termination method, with Sequenase (R) Version 2.0 (Cambridge Bioscience). The annealing step was carried out at 66°C for 2 mins, the labelling at 22°C for 4 mins, and the termination at 38°C for 5 mins. Areas containing secondary structure were resolved by the substitution of dITP for dGTG. Sequencing reactions were run on acrylamide gels 6% for 11, 8, 5 and 2 hrs at 46-51°C.

(vii) Subcloning with the TA cloning (RTM) system

The phagemid clone did not carry the 5' end of the cloned gene, therefore it was decided to subclone the 5' end by Polymerase Chain Reaction (PCR), followed by TA cloning (RTM). The following PCR reagents were added to each of the 2 tubes containing 0.0 µg, 0.7 µg of *S.sobrinus* MUCOB 263 genomic DNA, with distilled water to give 64.3 µl total volume; 5.3 µl or 0.1 µg/µl Primer 1 (CAGTCTCCGTCCCAACGACTGCG), 4.4 µl of 0.1 µg/µl Primer 2 (GCTCCTCTTGTGACATGGTC), 10 µl of 10 x Taq buffer (Northumbria Biochemicals Ltd., Northumberland (nbl), and 16 µl of dNTP's (12.5 µl of each of dATP, dGTP, dCTP, dTTP (Promega), added to 950 µl of deionized water). Then, 60 µl of mineral oil was carefully layered on top of the PCR reactions, the DNA denatured at 95°C for 10 mins, and 2.5 Weiss Units of Taq (nbl) added. The PCR conditions were 94°C for 1.5 mins, 50°C for 1.5 mins, and 72°C for 3 mins for 30 cycles, before a final long extension of 72°C for 10 mins. The PCR products (30 µl) were run on a TBE gel 0.8%, at 50 volts for 2 hours, alongside 4 µl of EcoR1 - Hind III cut lambda DNA markers (nbl).

Insert DNA was subcloned with the TA Cloning (RTM) System Version 1.3 (InVitrogen Corporation, British Biotechnologies Ltd, Oxon) as specified in the manufacturers protocol. Overnight, 12°C, ligation reactions containing 3.0 µl of a fourfold dilution, in distilled water, of the 0.7 µg PCR reaction, 4.7 µl of doubling dilution of the 0.7 µg PCR reaction, and a negative control were performed. In the transformation, white recombinant colonies were picked after overnight 37°C incubation, and twice replated on kanamycin (50µg/ml)/ 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal-25 µl of 40 mg/ml solution/plate) to achieve pure and stable subclones. Plasmid DNA was obtained, purified, denatured and sequenced. The origin of the cloned DNA from *S.sobrinus* was confirmed by Southern Blotting. The full

sequence obtained in shown in Table 4.

5.2 S.oralis cloning

This was done essentially by the methods described above. The differences were that the vector was lambda gt11 and the DNA source was a clinical isolate of Streptococcus oralis. The library was a genomic DNA library partially digested with EcoR1. Ten positive clones were identified and one of these subcloned by the TA Cloning (RTM) System. The origin of the clone from S.oralis was confirmed by Southern Blotting. Antigen directed antibody selection showed that it had epitopes which cross-reacted with the 180 KDa antigen of S.oralis (Figure 7). Tracks 1 and 3 show the original serum from two patients with antibody against S.oralis, and tracks 2 and 4 show the eluted subcomponent binding to the band at 180kDa. The sequence of the clone is given in Table 5.

Comparison of the Sequences

The sequence obtained from S.sobrinus had a 99.2% homology to the SpaA antigen of S.sobrinus and a 68.4% homology to the PAc antigen of S.mutans. The S.oralis peptide carried a 76.2% homology over 605 amino acids with the S.mutans PAc protein precursor and 73.8% homology over 606 amino acids with both the S.sobrinus SpaA protein precursor and the S.mutans surface antigen I/II precursor. Both sequences contain a three tandem repeat motif of 39 amino acids. Six peptides were derived from these and used to epitope map this communal sera.

Epitope Mapping

Epitope mapping of the antigen cloned from *S. oralis* was carried out according to the protocol outlined by Geysen *et al.*, J. Immunol. Methods, 102: 259-274 (1987) and references therein. In this epitope mapping, a complete set of overlapping nonapeptides was synthesised for the cloned *S. oralis* antigen. Peptide 1 covered residues 1-9, peptide 2 covered residues 2-10 and peptide 3 covered residues 3-11 etc. The epitopes were tested against various patient sera. All of the sera were examined at a 1 in 200 dilution for IgG and recording was stopped after 30 minutes. The sera examined were:

- (1) Viridans endocarditis (n=8) (*S. sanguis* n=2, *S. oralis* n=2, *S. gordonii* n=4)
- (2) *S. oralis* septicaemia n=5
- (3) *S. mutans* endocarditis (n=2)
- (4) *E. faecalis* endocarditis (n=2)
- (5) Negative control (n=5)

Epitopes were defined as those peptides which:

- (1) were positive in at least 3 wells
- (2) had an optical density of each well which was at least double the negative control value and in the majority of wells greater than 0.8

This epitope mapping identified a total of 9 epitopes (see table 10) - NFKQGQG, RQPG, SWYGAG, GKIRAV, RLFAQPQ, AGRPK, PTGYQFD, YPTVV and LLKKA.

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After the initial epitope mapping, each individual serum was then reexamined for positivity with each well (see table 11).

6. Preparation of Immunodominant Epitopes from Cloned *S.oralis*

Of the synthetic peptides (see 5.2; "Comparison of the Sequences"), peptides 1-4 cover the sequence of *S.oralis* as short peptides of 15 amino acid length. Peptides 5 and 6 covered the *S.sobrinus* area which differed. These sequences are listed (Table 6) and illustrated (Figure 8).

The six synthetic peptides were produced by Cambridge Biochemicals Ltd., Nantwich and dissolved in a double-distilled water to give a final concentration of 2 mg/ml. Aliquots, 0.5 ml, were stored at -70°C and after one freeze-thaw cycle, stored at -20°C.

The wells of six plastic microtitre plates (Falcon (R) 3912, Microtest (RTM) Flexible Assay Plate, 96 flat-bottom wells - Becton Dickinson & Co, Fred Baker, Liverpool), one for each peptide, were coated with 200 µl of the appropriate 10 µg/ml synthetic peptide solution diluted in phosphate buffered saline (one tablet dissolved in 100 ml distilled water, Oxoid, Unipath Ltd, Basingstoke; PBS). The peptides were added and left at 4°C overnight to coat the wells. Excess peptide was then removed by washing the microtitre plates five times in PBS with a Nunc-Immuno Wash (RTM) (InterMed, Denmark). The primary antibody, 200 µl of a 1/10 dilution in 3% BSA of sera was added and left at room temperature for 1 hour to allow antigen-antibody binding. After washing five times in PBS, the wells were filled with 200 µl of one of

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- i) a 1/1000 dilution of goat anti-human IgM peroxidase conjugate in 3% BSA (Sigma),
- ii) a 1/1000 dilution of goat anti-human IgG peroxidase conjugate in 3% BSA (Sigma), or
- iii) with PBS.

The conjugates were left to react at room temperature for 1 hour. The plates were then washed as before and 200 µl of ABTS stain was added to each well (three tablets of 2,2' amino-bis(3-ethylbenzthiazoline-6- sulfonic acid); diammonium salt; Sigma) and 160 µl of 30% w/w hydrogen peroxide solution; Sigma, in 60 ml of 125 mM disodium hydrogen orthophosphate buffer brought to pH 4.0 with 1M citric acid). This ABTS stain was prepared just prior to use. The signal strength for each well was then read at 405 nm at 5, 10 and 15 mins after the start of the reaction with a microtitre plate reader (Titertek Muliskan (10) PLUS MKH, Labsystems, Finland) attached to an impact dot matrix printer (Panasonic KX-P1081, Panasonic Matsushita Electric Industrial Co Ltd, Osaka, Japan).

To determine which of the six peptides representing the S.sobrinus/S.oralis antigenic region which produced the most specific and strongest positive signal, each of the peptides was used in an indirect ELISA.

All peptides were screened against a panel of 5 sera (single sera from cases of S.mutans endocarditis, S.oralis endocarditis, S.oralis septicaemia, S.lactis endocarditis and S.aureus endocarditis).

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All 6 peptides were recognised by the sera from the cases of endocarditis due to S.mutans and S.oralis. They had much lower Optical Densities with the sera from the septicaemia due to S.oralis and the control sera from cases of endocarditis due to S.lactis and S.aureus. Peptide 1 showed the highest OD (IgG) in S.mutans endocarditis and was selected for further studies (Table 7).

Indirect ELISA

As a result of the epitope mapping and of the preparation of immunodominant epitopes from clones S.oralis (see above), three peptides were investigated further in indirect ELISA tests. These peptides were YEVEKPLEPAPVAP (Peptide 1), TMYPNRQPGSGWDSS (contains the epitope RQPG - epitope numbers 74-79; Peptide 7) and WYSLNGKIRAVDVPK (contains the epitope GKIRAV - epitope numbers 144-147; Peptide 8).

Sera Tested

Sera from cases of endocarditis due to S.mutans (2), S.oralis (3), S.gordinii (10), S.sanguis (2), E.faecalis (11), S.bovis (8), S.agalactiae (1), S.lactis (1), S.pneumoniae (4), Group G Streptococcus (2), S.aureus (2), Coagulase Negative Staphylococci (6), Candida albicans (1), Candida parapsilosis (2) and E.coli (1), septicaemias due to S.oralis (8), E.faecalis (7), E.faecium (2) and a brain abscess due to S.milleri. Further controls were sera from patients with SLE (3) and neutropenic leukaemic patients with no evidence of streptococcal infection (20).

The optical densities are given in Table 8 for Peptide 1, and in Table 8a for Peptides 1, 2 and 3.

Results

In summary, a raised IgG was specific to patients with Streptococcal endocarditis and a raised IgM specific to endocarditis due to Streptococcus oralis/gordonii/sanguis/mitis.

If a cut off point of 0.6 is taken for the Optical Density for IgG and 0.4 for IgM then all cases of S.mutans (patient 1), S.oralis (patient 4), S.gordonii (patients 6-14) and S.sanguis (patients 16 and 17) endocarditis fulfilled one or other of these criteria. All other sera were classified as negative. All controls were negative except for a raised IgG in cases of endocarditis due to E.faecalis, S.bovis and Group G streptococci (Table 9).

This data proves the value of the test in culture positive endocarditis. It could also be extended to cover culture negative cases.

One treatment of IgM fell in cases 6, 13 and 15 showing that a falling IgM was a marker of successful therapy.

An analysis of the overall results (see tables 12 and 13 below) showed that the tests performed using peptide 1 gave the most accurate and specific results (100% specificity), but were the least sensitive (50% sensitivity). Tests with the other two

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peptides were more sensitive, but less specific and gave more false positives. This suggests that although each of the peptides could be usefully used individually, a combination of tests using more than one peptide would allow for an overall test which was both highly sensitive and highly specific.

Table 12

	Peptide 1		Peptide 7		Peptide 8	
	IgM	IgG	IgM	IgG	IgM	IgG
True Positives	6	5	12	5	12	10
False Positives	0	1	28	15	25	21
True Negatives	42	41	14	27	17	21
False Negatives	6	7	0	7	0	2

(For raw data see table 8a)

Table 13

	IgM Peptides			IgG Peptides		
	1	7	8	1	7	8
Sensitivity	50%	100%	100%	41.5%	41.5%	83%
Specificity	100%	33%	40%	98%	64%	50%
Efficiency	89%	48%	53%	85%	59%	57%

7. Fibronectin Binding Studies

In order to test for fibronectin binding activity in the cloned proteins, each

of the peptides 1-6 was reacted in an indirect ELISA according to the previous protocol.

The indirect ELISA produced OD values greater than 2 for all six peptides regardless of whether fibronectin was added to the sandwich. This implied that the polyclonal serum (anti-fibronectin 0.5 mg/ml Sigma [F1509]) reacted with the peptides directly. This serum was then immunoblotted at a dilution of 1 in 40 and again after crossabsorbing, 100 µl of 1 mg/ml antibody, with 100 µl of 1 mg/ml of fibronectin (F2006 Sigma) at 37°C for 30 mins. This showed that the antiserum reacted specifically with the 180 kDa antigen of S.oralis, 180 kDa antigen of S.gordonii and 120 kDa antigen of S.sanguis. This is illustrated by Figure 2 tracks 1 and 2 (S.gordonii), Figure 4 tracks 1 and 2 (S.oralis) and Figure 5 tracks 1 and 2 (S.sanguis). Figure 9 shows the antibody crossreacting with the 185 kDa antigen of S.sobrinus (Track 1) and the 180 kDa antigen of S.oralis (clinical isolate) (Track 3) and the elimination of the crossreactivity by prior absorption with fibronectin (Tracks 2 and 4).

This is with the previously identified immunodominant antigens of these microorganisms and implies that they act as a mimic for fibronectin. This appears to be the mechanism by which these streptococci bind to heart valves. A comparison of the sequences of the peptides with human fibronectin showed substantial homology (GCG programme Gap; Sequence of fibronectin by Kornblitt *et al.* EMBO J. 4: 1755-1759, 1985). The most homologous region of the fibronectin molecule was a 69 residue sequence beginning at residue 750 of the fibronectin protein. The percentage similarity and identity values were 48.7% and 35.9% respectively (Figure 10).

8. Human Recombinant Antibodies

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A library of immunoglobulin heavy and light chain variable (V) genes was prepared from the peripheral blood lymphocytes of a patient with infection due to a viridans streptococcus (blood culture positive) septicaemia following an oesophagectomy who recovered on amoxicillin and gentamicin. Screening of this library by immunoblot against S.oralis NCTC 7864 showed recombinant antibody against the band at 180 KDa (Figure 11, tracks 5 and 6). The pre and post IgM (Tracks 1 and 2) and IgG (Tracks 3 and 4) from a patient recovering from septicaemia due to S.oralis is shown for comparison.

The library was produced essentially as described by Marks *et al* (J. Mol. Biol., 1991, 222: 581-597) using the pCANTAB 5 vector, which is now commercially available as part of a kit from Pharmacia (Milton Keynes, UK). The heavy and light chain V genes, obtained from cDNA prepared from the mRNA of peripheral blood lymphocytes of a patient recovering from a viridans streptococcus (blood culture positive) septicaemia, were randomly combined and subcloned into Not I/Sfi I digested pCANTAB 5. The resulting single chain Fv fragments (ScFv), expressed on the surface of phage, were enriched by panning four times against the specific synthetic peptide epitopes YEVEKPLEPAPVAPS, TMYPNRQPGSGWDSS and WYSLNGKIRAVDVPK (Peptides 1, 7 and 8). Following the fourth panning, twenty clones (from each of the last pannings) were:

1. Bst1 fingerprinted to establish the degree of focussing of the panning procedure; and
2. Examined in an indirect ELISA against the original peptide (as described

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in detail previously). Conditions were:-

Recombinant antibody: neat

Phage specific monoclonal : 1 in 2,000

Antimouse Horse Radish Peroxidase 1 in 1,000

100ml of Peptide applied to well in PBS at 10ug/ml and incubated overnight at 4°C.

Results

Peptide 1

20 clones selected were identical on BstI fingerprinting. Indirect ELISA varied from 0.25-0.30 (control 0.17). Two clones (PAC 1 and PAC 2) selected for animal work.

Peptide 7

16 clones produced 6 types. One type (Type A) was present in 7 out of the 16 clones and was the only type to produce a positive reading in the indirect ELISA (0.276-0.318) (control 0.17). One clone (Clone 3) selected for animal work (PAC 3).

Peptide 8

16 clones produced 10 types. Two of these, A and B were represented by 3 and 5 clones respectively. None of these clones produced a positive ELISA result. Five of the 8 clones produced a positive ELISA result (range 0.235-0.304 control 0.138) and a unique BstI fingerprint. One of these (Clone 7) was selected for animal work (PAC 4).

In order to test the efficacy of the selected clones specific to Peptides 1-3 in treating *S. oralis* and vancomycin-resistant *E. faecium* infections, a set of experiments with PAC 1-4 and other controls was performed.

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Experiment 1

This experiment was an acute streptococcal infection model using Balb/c mice with an end point of death. Conditions were:

S.oralis dose: 5.7×10^9

Phage dose: 5×10^8 pfu/ml

Antibody given first, followed by *S.oralis* 2 hours later.

Table 14 (results of Experiment 1)

SURVIVORS				
Antibody	No. of mice	4 hours	24 hours	48 hours
Control: no antibody M13K07 phage	15	7	3	1
DEPAGE	13	3	3	2
B3.7	13	6	6	6
B3.14	8	1	1	1
PAC 1	9	9	2	2
PAC 2	17	8	8	8
PAC 3	15	6	6	6
PAC 4	15	2	2	2

DEPAGE - antibody against a Candidal specific carboxy-end HSP90 antigen (sequence DEPAGE) which acts as an irrelevant phage and therefore a control.

Fisher exact 2 tailed P value showed statistical significance for B3.7 at 48 hours (P 0.03), PAC 1 at 2 hours (P 0.0095) and PAC 2 at 48 hours (P 0.02). B3.7 is a recombinant

antibody specific to the HSP90 stress protein, suggesting that antibodies specific to the HSP90 protein may be used to diagnose and treat *S. oralis* and possibly other streptococcal and enterococcal infections.

Experiment 2

This was a chronic streptococcal infection model using CD1 mice and a colony count of spleen and kidney was performed at the various end-points. Conditions were:

S.oralis dose 2.5×10^9

Phage dose 5×10^{10} pfu/ml

S.oralis given first, antibody given 24 hours later.

Positive organ count $> 10^4$ /g/ml

S = Spleen, K = Kidney

S + K = combined spleen and kidney results

5 mice sacrificed on days 4, 7 and 12

Repeat antibody injection at day 10.

Table 15 (results of Experiment 2)

	Day 4			Day 7			Day 12		
	S	K	S+K	S	K	S+K	S	K	S+K
Control:no antibody M13K07 phage	4	4	8	0	0	0	1	0	1
B3.14	1	2	3	0	0	0	0	0	0
PAC 2	0	1	1	1	1	2	1	0	1
PAC 3	1	2	3	0	0	0	0	0	0

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No phage recovered after 24 hours post injection, all mice blood cultures negative.

Fisher exact 2 tailed P value showed statistical significance for PAC 2 ($P = 0.005$) when spleen and kidney results were combined from day 4.

Experiment 3

This was an infection model with CD1 mice using high level vancomycin-resistant *E. faecium*. Conditions were:

E. faecium dose: 3×10^{10} ml

Phage dose: 5×10^{10} pfu/ml

E. faecium given first, antibody given 24 hours later.

Positive $> 10^4$ g/ml. S = spleen, K = Kidney

S + K = Spleen + Kidney.

Spontaneous deaths cultured at day 2

5 mice sacrificed on day 4

Remaining mice 3 for the M13K07 control and 5 for the PAC 2 antibody, sacrificed at day 7.

Table 16 (results of Experiment 3)

	Day 2 (spontaneous deaths)	Day 4 (n=5)			Day 7 (n=3 control) (n=5 PAC 2)		
		S	K	S+K	S	K	S+K
Control no antibody: M13K07 phage	6 ^a	2	5	7	0	0	0
PAC 2	4 ^a	2	3	5	0	0	0

^a all spleens and kidneys cultured and positive > 10⁴ /gl/ml

Total positive Control : 19/22

PAC : 13/18

This produced by combing the spleen and kidney results obtained from the spontaneous deaths (day 2) with 5 mice (day 4) sacrificed assuming > 10⁴ organisms /g/ml is positive. This suggests PAC2 may have some activity agianst vancomycin-resisitant *E.faecium*.

Experiment 4

This was an acute streptococcal infection model using Balb/c mice infected with vancomycin-resistant *E. faecium*. The end-point was death. Coonditions were:

Organism: high level (>256 mg/l) vancomycin-resistant *E. faecium*

E. faecium dose: 5 x 10¹⁰ /ml

Phage dose: 5 x 10¹⁰ pfu /ml

Antibody given first, followed by *E.faecium* 2 hours later.

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Table 17 (results of experiment 4)

Antibody	No of mice	Survivors		
		4hours	24hours	48hours
Control no antibody M13K07 phage	15	10	5	1
PAC2	15	12	8	6

Fisher exact 2 tailed P value showed statistical significance for Pac 2 at 48 hours (p 0.02)

Table 1 Immunoblot testing of the *S.oralis* NCTC 7864 antigen against patient sera.

M.Wt. of <i>S.oralis</i> antigen band	Patient Sera						
	Endocarditis	Sequential sera <i>S.oralis</i> . septicaemia			Controls		
	n = 12						
	<i>S.oralis</i> Endo- carditis	<i>S.gordonii</i> Endo- carditis	<i>S.sanguis</i> Endo- carditis	Constant Septicaemia	Inc. x 2 or App.	Controls Neutropenic	Controls Non- Neutropenic
	n = 2	n = 4	n = 2			n = 20	n = 20
	IgM IgG	IgM IgG	IgM IgG	IgM IgG	IgM IgG	IgM IgG	IgM IgG
192	1 1	2 10	1 2	1 3	1 5	4 6	2 1
185	1	4	1 1	1	1 3	2 3	1 2
180	2 2	4 13	1 2	2	9 10	1 3	4
155	1	9	1	4		2	
140	1 2	2 6		3 8	1 2	2 8	1 2
105	1	1 5	1	1		2 2	1 1
85	2 2	4 11	1 2	8	1 1	3 3	1 1
76	1	1 3	1	1 5	2 1	2 2	
65	1	3			1		
51	1	6 3	1	11	2	1 11	6
46	2	3		1	1	1	
35	1	1	1				

Inc = Increased x 2

App = Appeared

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Table 2 Immunoblot testing of the *S.gordonii* NCTC 7868 antigen against patient sera.

M.Wt. of <i>S.oralis</i> antigen band	Patient Sera						
	Endocarditis		Sequential sera <i>S.oralis</i> . septicaemia n = 12			Controls	
	<i>S.oralis</i> Endo- carditis	<i>S.gordonii</i> Endo- carditis	<i>S.sangius</i> Endo- carditis	Constant Septicaemia	Inc. x 2 or App.	Controls Neutropenic	Controls Non- Neutropenic
	n = 2	n = 4	n = 2			n = 20	n = 20
	IgM IgG	IgM IgG	IgM IgG	IgM IgG	IgM IgG	IgM IgG	IgM IgG
185	2	2 5		1 7			
180	2	12 14	2 2	6		5	1 2
105	1	1		2 4		1 3	1
85	2 1	12 14	1 1	1 10			1 1
80		5 12	1 1	1 9		1 11	4
76		5 7	1 1			1 10	1 3
72	1 1	5 7	1		1	1 4	1 1
70	1	4 5	1 2	7			
65		9 13	1 2	1 4		1	1
58		1 9					
56		2 9	1				
52		1 8	2				
47	2	12 14	1 1	10	2	1 15	2 2
40		5 8	2 1	8	1	1	
37		8 8	2 2				
35		3 10	1 1	2	1		
33		2 9					
30		1 11	2 2				
28		2 7	1				

Inc = Increased

App = Appeared

Table 3 Immunoblot testing of the *S.sanguis* NCTC 7863 antigen against patient sera.

M.Wt. of <i>S.sanguis</i> antigen band	Patient Sera					
	Endocarditis			Septicaemia		
	<i>S.oralis</i> Endo- carditis	<i>S.gordonii</i> Endo- carditis	<i>S.sanguis</i> Endo- carditis	<i>S.oralis</i> Septicaemia	Controls Neutropenic	Controls Non- Neutropenic
	n = 2	n = 14	n = 2	n = 9	n = 20	n = 20
	IgM IgG	IgM IgG	IgM IgG	IgM IgG	IgM IgG	IgM IgG
120	2	4 14	2 2	1	3 3	4
85	1	2	2 2	8	3 3	2 4
83			2 2			
81		2 8	2 2			
75	1 1	4 2	2	2 5	3 15	8
59	1 1	2	1			2 2
57	1 2	2 12	1			2 2
55	1 1		2		6	2 12
50				9		2
47		2	2 2		9	2 6
42	1	6	2 2		9	
40			2 2	9		2
37				3		2 2
35			1			
32						
25		6				2

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Table 4 Full sequence of cloned *S.sobrinus* gene

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5'          10          30          50
GCTCCTCTTG TGACATGGTC ATAGTAACAG ATAATCTGTT TAATTTCAAG CAGATTTAAT

          70          90          110
AGCCTCCAGG AACTTGAAA TAAAACTGAA ATAAAACTGA ATTTTTTATA AAGCCTAGAT

          130          150          170
TAAGCAATCG TTTGCATTGA CAATCACTAG ATAAGTGTTA TTATAGATAG TATTGTAACG

          190          210          230
AAACATTTCG GATGTTACAA AAATGTAAAT TGGAGGGAAT TATAATATGC AACGAAAAGA
                                   M   Q   R   K   E

          250          270          290
GACTTTTGGG TTTCGCAAAA GTAAATCAG TAGGACCCTT TGTGGTGCCT TACTAGGAAC
      T   F   G   F   R   K   S   K   I   S   R   T   L   C   G   A   L   L   G   T

          310          330          350
TGCTATCTTA GCGTCTGTAA CAGGTCAAAA GCGCCTCGCT GAAGAAACAA GTACCACTTC
      A   I   L   A   S   V   T   G   Q   K   A   L   A   E   E   T   S   T   T   S

          370          390          410
AACTTCGGGG GTTAATACCG CAGTCGTTGG GACGGAGACT GGAATCCCG CCACCAACCT
      T   S   G   V   N   T   A   V   V   G   T   E   T   G   N   P   A   T   N   L

          430          450          470
GCCTGACAAA CAGGACAATC CAAGTTCGCA AGCCGAGACA AGTCAGGCCC AAGCCGGTCA
      P   D   K   Q   D   N   P   S   S   Q   A   E   T   S   Q   A   Q   A   G   Q

          490          510          530
AAAGACAGGG GCAATGTCAG TAGATGTGTC TACAAGTGAG CTTGACGAAG CTGCTAAAAG
      K   T   G   A   M   S   V   D   V   S   T   S   E   L   D   E   A   A   K   S

          550          570          590
TGCCCAAGAA GCTGGTGTGA CCGTTTCGCA GGATGCTACC GTCGATAAAG GGACAGTAGA
      A   Q   E   A   G   V   T   V   S   Q   D   A   T   V   D   K   G   T   V   E

          610          630          650
AACTTCTGAC GAAGCTAACC AAAAAGAAAC CGAAATCAAG GATGACTACA GCAAGCAAGC
      T   S   D   E   A   N   Q   K   E   T   E   I   K   D   D   Y   S   K   Q   A

          670          690          710
AGCAGACATC CAAAAGACAA CAGAAGACTA CAAGGCAGCT GTGGCTCGTA ACCAAGCCGA
      A   D   I   Q   K   T   T   E   D   Y   K   A   A   V   A   R   N   Q   A   E

          730          750          770
AACAGACCGA ATCACTCAAG AAAACGCGGC TAAGAAGGCC CAATACGAAC AAGATTGCGC
      T   D   R   I   T   Q   E   N   A   A   K   K   A   Q   Y   E   Q   D   L   A

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790 810 830
 GGCCAACAAG GCAGAAGTGG AACGCATTAC CAATGAGAAT GCGCAACGCA AGGCTGATTA
 A N K A E V E R I T N E N A Q R K A D Y

850 870 890
 CGAAGCTAAG CTGGCTCAAT ATCAAAAGGA CCTAGCAGCC GTTCAACAAG CTAATAATGA
 E A K L A Q Y Q K D L A A V Q Q {A N N D
 A1

910 930 950
 CAGTCAAGCA GCCTACGCTG CTGCCAAGGA AGCCTACGAC AAAGAATTGG CTCGGGTTCA
 S Q A A Y A A A K E A Y D K E L A R V Q

970 990 1010
 AGCTGCTAAT GCCGCTGCTA AGAAAGAATA CGAAGAGGCT CTAGCTGCCA ACACCACTAA
 A A N A A A K K E Y E E A L A A N T T K

1030 1050 1070
 GAATGAGCAA ATCAAGGCAG AAAACGCCGC TATCCAGCAA CGCAATGCCC AAGCTAAGGC
 N E Q I K A E N A A I Q Q R N A Q A K A

1090 1110 1130
 AGATTACGAA GCCAAGTTGG CTCAATATGA AAAAGATTTA GCCGCAGCCC AGTCTGGTAA
 D Y E A K L A Q Y E K D L A A A Q S}{G N
 A2

1150 1170 1190
 TGCTACAAAT GAAGCGGACT ACCAAGCTAA GAAGGCAGCT TATGAACAAG AGTTAGCGCG
 A T N E A D Y Q A K K A A Y E Q E L A R

1210 1230 1250
 CGTGCAAGCC GCTAATGCAG CTGCCAAGCA GGCCTACGAA CAAGCTCTAG CTGCCAACAC
 V Q A A N A A A K Q A Y E Q A L A A N T

1270 1290 1310
 GGCCAAGAAC GCCCAAATCA CGGCCGAAAA TGAGGCTATC CAGCAGCGCA ATGCGCAAGC
 A K N A Q I T A E N E A I Q Q R N A Q A

1330 1350 1370
 TAAGGCTAAC TATGAAGCTA AATTAGCCCA ATATCAAAAG GATTTGGCCG CAGCTCAATC
 K A N Y E A K L A Q Y Q K D L A A A Q S}

1390 1410 1430
 TGTAACGCC GCTAATGAGG CAGACTACCA AGAAAAATTA GCAGCCTATG AAAAGGAACT
 {G N A A N E A D Y Q E K L A A Y E K E L
 A3

1450 1470 1490
 GGCTCGTGTG CAAGCAGCCA ATGCAGCTGC TAAGCAAGAA TATGAGCAGA AAGTTCAGGA
 A R V Q A A N A A A K Q E Y E Q K V Q E

1510 1530 1550
 AGCTAATGCT AAAAATGCCG AAATTACGGA AGCCAACCGT GCTATCCGTG AACGCAATGC
 A N A K N A E I T E A N R A I R E R N A

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1570	1590	1610
CAAGGCCAAG ACAGACTATG AACTCAAAC	GTCTAAGTAC	CAAGAAGAGC TTGCTCAGTA
K A K T D Y E L K L	S K Y	Q E E L A Q Y
1630	1650	1670
CAAGAAGGAC CTAGCGGAAT ACCCAGCTAA	ACTCCAAGCC	TATCAAGATG AACAAAGCCGC
K K} D L A E Y P A K	L Q A	Y Q D E Q A A
1690	1710	1730
AATCAAGGCA GCTCTGGAAG AGTTGGAAAA	GCACAAGAAT	GAAGATTGGA ACCTCAGTGA
I K A A L E E L E K	H K N	E D W N L S E
1750	1770	1790
GCCCTCAGCC CAGAGTCTGG TCTATGACTT	GGAGCCCAAT	GCTCAGATTT CCCTAGTGAC
P S A Q S L V Y D L	E P N	A Q I S L V T
1810	1830	1850
CGATTGGAAG CTACTGAAAG CCTCCTCCCT	TGATGAATCC	TTTAGCCACG ATACTGAACA
D W K L L K A S S L	D E S	F S H D T E Q
1870	1890	1910
ATATAACAAA CACAACCTGC AGCCAGATAA	TCTAAATATA	ACCTATCTGG AGCAGGCTGA
Y N K H N L Q P D N	L N I	T Y L E Q A D
1930	1950	1970
TGATGTGGCC TCCTCAGTAG AGCTCTTTGG	TAATTTTCGGT	GATAAGGCTG GTTGGACAAC
D V A S S V E L F G	N F G	D K A G W T T
1990	2010	2030
CACTGTCAGC AATGGTTCAG AAGTTAAGTT	TGCCTCTGTC	CTCCTCAAGC GTGGCCAGAG
T V S N G S E V K F	A S V	L L K R G Q S
2050	2070	2090
TGCTACAGCC ACCTATACCA ACCTGAAAAA	CTCTTACTAC	AATGGTAAGA AAATTTCTAA
A T A T Y T N L K N	S Y Y	N G K K I S K
2110	2130	2150
GGTGGTCTAC AAGTATACGG TTGACCCTGA	CTCCAAGTTT	CAAAATCCTA CTGGTAACGT
V V Y K Y T V D P D	S K F	Q N P T G N V
2170	2190	2210
TTGGTTAGGT ATCTTTACTG ACCCAACCCT	AGGGGTCTTT	GCCTCAGCCT ATACGGGTCA
W L G I F T D P T L	G V F	A S A Y T G Q
2230	2250	2270
AAACGAGAAG GATACCTCTA TCTTTATCAA	GAATGAATTC	ACCTTCTACG ATGAAGACGG
N E K D T S I F I K	N E F	T F Y D E D G
2290	2310	2330
TAATCCCATC GACTTTGATA ATGCCCTCTT	GTCAGTTGCC	TCCCTTAACA GGGAACACAA
N P I D F D N A L L	S V A	S L N R E H N

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2350 2370 2390
TTCCATTGAG ATGGCCAAGG ACTACAGCGG TACCTTCGTT AAGATTTCTG GCTCATCCAT
S I E M A K D Y S G T F V K I S G S S I

2410 2430 2450
TGGTGAAAAA AATGGCATGA TCTATGCGAC CGACACCCTC AACTTTAAAA AGGGTGAAGG
G E K N G M I Y A T D T L N F K K G E G

2470 2490 2510
CGGTTCCCTT CACACCATGT ACACCAGAGC AAGTGAGCCT GGTTCAGGTT GGGACTCTGC
G S L H T M Y T R A S E P G S G W D S A

2530 2550 2570
TGATGCTCCT AATTCTTGGT ATGGTGCTGG TGCTGTCAGA ATGTCCGGCC CAAACAATA
D A P N S W Y G A G A V R M S G P N N Y

2590 2610 2630
CATCACTTTG GGGGCAACCT CAGCGACCAA TGTCCTCAGC CTAGCTGAAA TGCCACAGGT
I T L G A T S A T N V L S L A E M P Q V

2650 2670 2690
ACCTGGTAAA GATAATACTG CTGGTAAAAA ACCAAATATC TGGTATTCCC TTAATGGTAA
P G K D N T A G K K P N I W Y S L N G K

2710 2730 2750
GATTCGGGCA GTCAATGTCC CTAAAGTGAC CAAGGAAAAA CCAACCCAC CAGTTGAGCC
I R A V N V P K V T K E K P T P P V E P

2770 2790 2810
AACCAAGCCA GACGAGCCAG TCTATGAAGT TGAGAAGGAA TTGGTAGATC TGCCAGTTGA
T K P D E P V Y E V E K E L V D L P V E

2830 2850 2870
ACCAAGCTAC GAAAAGGAAC CAACCCACCC AAGCAAGACT CCAGACCAAA ATATCCCAGA
P S Y E K {E P T P P S K T P D Q N I P D
P1

2890 2910 2930
CAAACAGTA GAGCCTACTT ATGAGGTTGA AAAGGAGCTG GAACCGGCAC CAGTTGAGCC
K P V E P T Y E V E K E L E P A P V E P

2950 2970 2990
AAGCTACGAA AAGGAACCAA CGCCACCAAG CAAGACTCCG GATCAAGCGA TTCCAGACAA
S Y E K {E P T P P S K T P D Q A I P D K
P2

3010 3030 3500
ACCGGTAGAG CCAACCTATG AGGTTGAAAA GGAGTTGGAA CCAGTACCTG TAGAAACAAA
P V E P T Y E V E K E L E P V P V E T N

- 52 -

3070 3090 3110
 CTACGAAAAG GAACCAACCC CGCCTCAGTC AACCCCAGAC CAAGAAGAGC CCACCAAACC
 Y E K} {E P T P P Q S T P D Q E E P T K P
 P3

3130 3150 3170
 GGTGGAACCA AGCTACCAAA GCTTGCCAAC CCCACCAGTG GCACCGACTT ATGAAAAGGT
 V E P S Y Q} S L P T P P V A P T Y E K V

3190 3210 3230
 TCCTGGTCCT GTCAGTGTGC CAACGGTTCG GTACCACTAC TATAAACTAG CGGTCCAACC
 P G P V S V P T V R Y H Y Y K L A V Q P

3250 3270 3290
 CGGCGTCACC AAGAAAATCA AAAACCAGGA TGACCTGGAT ATTGACAAGA CCCTGGTGCC
 G V T K K I K N Q D D L D I D K T L V A

3310 3330 3350
 TAAGCAGTCG ACGGTTAAGT TCCAATTGAA GACAGCAGAC CTGCCAGCCG GTCGTCCAGA
 K Q S T V K F Q L K T A D L P A G R P E

3370 3390 3410
 AACGACCTCC TTTGTCTTGA TGGATCCTCT GCCAAGCGGT TACCAACTTA ATCTGGAAGC
 T T S F V L M D P L P S G Y Q L N L E A

3430 3450 3470
 TACCAAGGTC GCCAGCCCAG GCTTTGAAGC TAGCTATGAT GCCATGACCC ATACGGTAAC
 T K V A S P G F E A S Y D A M T H T V T

3490 3510 3530
 CTTCATCGCA ACCGCTGAGA CCTTGGCGGC GCTCAACCAG GATCTGACCA AGGCCGTGGC
 F I A T A E T L A A L N Q D L T K A V A

3550 3570 3590
 GACTATCTAC CCAACAGTTG TGGGACAAGT CCTCAACGAT GGCGCTACCT ACACCAATAA
 T I Y P T V V G Q V L N D G A T Y T N N

3610 3630 3650
 CTTACCCCTG ATGGTCAATG ATGCTTACGG TATTAAATCC AATATCGTTC GCGTGACCAC
 F T L M V N D A Y G I K S N I V R V T T

3670 3690 3710
 ACCAGGGAAA CCTAACGACC CAGACAACCC AAGCAACAAC TACATCACCC CGCACAAGGT
 P G K P N D P D N P S N N Y I T P H K V

3730 3750 3770
 CAAVAAGAAT GAAAACGGTG TGGTGATTGA TGGTAAGTCC GTCCTAGCTG GTACCACCAA
 N K N E N G V V I D G K S V L A G T T N

3790 3810 3830
 CTACTATGAA TTGACTTGGG ACCTGGACCA ATACAAGGGC GATAAATCGG CCAAGGAGAC
 Y Y E L T W D L D Q Y K G D K S A K E T

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3850 3870 3890
CATCCAAAAA GGCTTCTTCT ATGTGGATGA CTATCCTGAA GAAGCGCTGG ACTTGCGCAC
I Q K G F F Y V D D Y P E E A L D L R T

3910 3930 3950
CGACCTGATT AAGCTGACCG ATGCCAACGG CAAGGCGGTC ACTGGTGTCA GCGTGGCTGA
D L I K L T D A N G K A V A G V S V A D

3970 3990 4010
CTACGCCAGT CTGGAGGCCG CACCAGCAGC TGTTCAGAC ATGCTCAAGA AGGCCAACAT
Y A S L E A A P A A V Q D M L K K A N I

4030 4050 4070
TACCCCTAAG GGAGCCTTCC AAGTCTTTAC CGCTGACGAT CCTCAGGCCT TCTACGATGC
T P K G A F Q V F T A D D P Q A F Y D A

4090 4110 4130
CTATGTGGTT ACCGGAAGT ACCTGACCAT CGTCACTCCA ATGACGGTCA AGGCTGAGAT
Y V V T G T D L T I V T P M T V K A E M

4150 4170 4190
GGGCAAGATC GGTGGTAGCT ATGAAAACAA GGCCTACCAG ATTGACTTTG GTAATGGCTA
G K I G G S Y E N K A Y Q I D F G N G Y

4210 4230 4250
TGAATCTAAT ATTGTGATTA ACAATGTGCC GCAAATCAAT CCTGAAAAGG ATGTGACCTT
E S N I V I N N V P Q I N P E K D V T L

4270 4290 4310
GACCATGGAT CCAGCGGATA GTACCAATGT GGATGGACAG ACCATCGCCC TCAATCAGGT
T M D P A D S T N V D G Q T I A L N Q V

4330 4350 4370
CTTTAACTAC CGTCTCATCG GTGGTATCAT TCCAGCGGAC CATGCCGAAG AGCTCTTTGA
F N Y R L I G G I I P A D H A E E L F E

4390 4410 4430
GTACAGCTTT AGCGATGACT ATGACCAAAC TGGAGACCAG TACACGGGCC AATACAAGGC
Y S F S D D Y D Q T G D Q Y T G Q Y K A

4450 4470 4490
CTTTGCCAAG GTTGACCTGA CCCTCAAGGA TGGTACAATC ATCAAGGCGG G TACTGACTT
F A K V D L T L K D G T I I K A G T D L

4510 4530 4550
GACTTCATAT ACAGAAGCGC AAGTTGATGA AGCTAATGGC CAAATTGTTG TGACCTTCAA
T S Y T E A Q V D E A N G Q I V V T F K

4570 4590 4610
GGAAGATTTT TTGCGGTCTG TGTCTGTAGA CTCGGCCTTC CAAGCGGAAG TCTACCTACA
E D F L R S V S V D S A F Q A E V Y L Q

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4630 4650 4670
GATGAAGCGG ATAGCCGTCG GGACCTTTGC CAATACCTAT GTCAATACGG TCAATGGAAT
M K R I A V G T F A N T Y V N T V N G I

4690 4710 4730
TACCTATAGC TCTAATACGG TAAGGACCAG CACACCAGAG CCGAAGCAGC CAAGTCCAGT
Y Y S S N T V R T S T P E P K Q P S P V

4750 4770 4790
GGTACCTAAG ACCACTACTA CGGTAGTCTT CCAGCCTCGT CAGGGTCAAG CTTATCAGCC
V P K T T T T V V F Q P R Q G Q A Y Q P

4810 4830 4850
AGCGCCGCCA GCAGGAGCTC AATTGCCAGC CACAGGGGAT AGTAGCAATG CTTACCTGCC
A P P A G A Q L P A T G D S S N A Y L P
- - -

4870 4890 4910
ACTTTTAGGC CTCGTAAGCC TGACTGCTGG CTTTAGCCTG TTAGGACTGC GCCGGAAGCA
L L G L V S L T A G F S L L G L R R K Q

4930 4950 4970
GGACTAAAGA ATCCAACAAG AAAAAATGGG AAAGTTTGCC TTTCTCATTT TTTATATTCC
D *

4990 5010 5030
CAGCTAGCTG AGTAGTCAAG AAGTACTCTT AGAAAACCCT AGAGAACATT AGCTAACTTT

5050 5070
TCCAAACCGA TAGACGTTTA TTTTAGTCTA AGTATGG

Table 5 The *S.oralis* DNA Sequence Encoding an Endocarditis - Specific Antigen Region

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5'          10          30          50
GAATTCACCT TCTACGATGA AAATGACCAA CCAATTAATT TTGACAATGC TCTTCTTTCA
E F T F Y D E N D Q P I N F D N A L L S

          70          90          110
GTAGCCTCAC TTAACCGTGA GCATAACTCT ATTGAGATGG CTAAGGATTA TAGTGGTACT
V A S L N R E H N S I E M A K D Y S G T

          130          150          170
TTTATTAAAA TCTCAGGTTC ATCCATCGGT GAAAAAATG GCATGATTTA TGCCACAGAA
F I K I S G S S I G E K N G M I Y A T E

          190          210          230
ACCCTGAACT TTAACAAGG ACAGGGTGGA GCTCGCTGGA CAATGTATCC AAATCGTCAG
T L N F K Q G Q G G A R W T M Y P N R Q

          250          270          290
CCAGGTTTCA GTTGGGATTC ATCAGATGCA CCAAACCTCTT GGTACGGTGC AGGGGCCATT
P G S G W D S S D A P N S W Y G A G A I

          310          330          350
AGTATGTCCG GTCCTACGAA TCACGTTACA GTTGGTGCAA CATCTGCTAC CAATGTGATG
S M S G P T N H V T V G A T S A T N V M

          370          390          410
TCCGTAGCAG AAATGCCTCA AGTACCTGGA AGAGACAATA CTGAAGGTAA AAGACCAAAC
S V A E M P Q V P G R D N T E G K R P N

          430          450          470
ATCTGGTACT CACTCAATGG TAAATTCGT GCGGTTGACG TTCCGAAAAT TACAAAAGAA
I W Y S L N G K I R A V D V P K I T K E

          490          510          530
AAACCAACTC CACCGGTAGC ACCAACTGAA CCACAAGCTC CTACCTATGA AGTGGAGAAA
K P T P P V A P T E P Q A P T Y E V E K

          550          570          590
CCACTGGAAC CGGCTCCAGT AGCACCAAGC TACGAAAATG AGCCAACCTCC ACCAGTAAAA
P L E P A P V A P S Y E N {E P T P P V K
                               P1

          610          630          650
ACTCCAGATC AACCGGAGCC ATCAAAACCA GAAGAGCCAA CATATGAGAC AGAGAAACCA
T P D Q P E P S K P E E P T Y E T E K P

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670 690 710
 TTGGAACCAG CTCCAGTAGC ACCAAACTAC GAAAATGAGC CAACTCCACC AGTAAAAACT
 L E P A P V A P N Y E N } { E P T P P V K T
 P2

730 750 770
 CCAGATCAAC CAGACCCATC AAAACCGGAA GAGCCAAACT ATGAGACAGA GAAACCATTG
 P D Q P D P S K P E E P N Y E T E K P L

790 810 830
 GAACCAGCTC CAGTAGCACC AAGCTATGAA AATGAGCCAA CTCCACCGGT AAAAAGCTCA
 E P A P V A P S Y E N } { E P T P P V K T P
 P3

850 870 890
 GATCAACCAG AGCCATCAAA ACCAGAAGAG CCAAATTATG ATCCATTGCC AACTCCGCCG
 D Q P E P S K P E E P N Y D } P L P T P P

910 930 950
 CTAGCACCAA CTCCTAAGCA GTTGCCAACA CCACCAGCGG TGCCAACAGT TCACTTCCAT
 L A P T P K Q L P T P P A V P T V H F H

970 990 1010
 TACAATCGTC TATTTGCACA ACCTCAGATT AATAAAGAAA TTAAAAACGA GGATGGAGTA
 Y N R L F A Q P Q I N K E I K N E D G V

1030 1050 1070
 GATATTGATC GTACTCTAGT TGCTAAGCAG TCTGTAGTGA AGTTTGAGCT GAAAACAGAA
 D I D R T L V A K Q S V V K F E L K T E

1090 1110 1130
 GCTTTAACTG CTGGTCGTCC AAAAACAAC TCGTTTGTAT TGGTAGATCC ACTTCCAAC
 A L T A G R P K T T S F V L V D P L P T

1150 1170 1190
 GGCTATCAGT TTGATTTGGA AGCAACCAAG GCTGCAAGCA AAGGTTTTGA AACAAAGCTAT
 G Y Q F D L E A T K A A S K G F E T S Y

1210 1230 1250
 GACAAAGCTA GTCACACTGT AACCTTTAAG GCTACTGAGG AGACCTTAGC TGCTTTCAAT
 D K A S H T V T F K A T E E T L A A F N

1270 1290 1310
 GCTGATTTGA CAAAATCCTT TGAGACTCTA TATCCAACCTG TTGTTGGTCG TGTCTTGAAT
 A D L T K S F E T L Y P T V V G R V L N

1330 1350 1370
 GATGGGGCGA CTTATACGAA TAACTTTACA TTGACAGTCA ACGATGCTAC TGGTGTCAAG
 D G A T Y T N N F T L T V N D A T G V K

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      1390                      1410                      1430
TCAAACATTG TTCGTGTAAC GACTCCAGGT AAACCAAATG ATCCTGACAA TCCAAATAAC
S N I V R V T T P G K P N D P D N P N N

      1450                      1470                      1490
AACTACATCA AGCCTTTGAA AGTTAACAAG AACAAGCAAG GTGTGAATAT TGATGGCAAA
N Y I K P L K V N K N K Q G V N I D G K

      1510                      1530                      1550
GAAGTTCTAG CTGGTTCAAC GAACTACTAT GAACTCACAT GGGATTTGGA TCAATACAAG
E V L A G S T N Y Y E L T W D L D Q Y K

      1570                      1590                      1610
GGAGATAAAT CTTCTAAAGA AGCGATTCAA AATGGTTTCT ACTATGTGGA TGATTATCCA
G D K S S K E A I Q N G F Y Y V D D Y P

      1630                      1650                      1670
GAAGAAGCTT TAACGCTTCA ACCAGAATTG GTTAAGATTG GTGATCTAGA GGGCAACCTT
E E A L T L Q P E L V K I R D L E G N L

      1690                      1710                      1730
GTATCAGGTA TCAGTGTTCA ACAGTTTGAT AGTTTAGAAC GTGCGCCTAA GAAGGTTCAA
V S G I S V Q Q F D S L E R A P K K V Q

      1750                      1770                      1790
GATCTGTTGA AGAAAGCAAA CATCACTGTT AAAGGTGCTT TCCAACCTCT CTCAGCTGAT
D L L K K A N I T V K G A F Q L F S A D

      1810
AATCCAGCTG AATTC
N P A E F

```

This 1.81 kb DNA fragment carried the three tandem repeat proline (P1 - 3) common to the family of antigen 1/11 proteins important in dental caries. The repeat proline rich region of this amino acid sequence was bracketed ({}) as above.

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Table 6

Peptide Number	Amino Acid Sequence
1	YEVEKPLEPAPVAPS
2	SYENEPTPPVKTPDC
3	KTPDQPEPSKPEEPT
4	EPAPVAPSYENEPTP
5	YEVEKELVDLPVEPS
6	KTPDQNIPDKPVEPT

Table 7

Clinical History of Serum

Peptide Number						
	1	2	3	4	5	6
	M G	M G	M G	M G	M G	M G
<i>S. mutans</i> endocarditis	0.341 1.506	0.331 0.381	0.338 1.365	0.445 1.225	0.261 1.491	0.241 0.869
<i>S. oralis</i> endocarditis	0.442 0.902	0.561 0.891	0.491 0.768	0.567 0.936	0.653 0.784	0.373 0.873
<i>S. oralis</i> septicaemia	0.024 0.118	0.036 0.215	0.019 0.123	0.065 0.168	0.043 0.241	0.117 0.218
<i>S. lactis</i> endocarditis	0.306 0.589	0.319 0.528	0.309 0.492	0.377 0.415	0.337 0.421	0.244 0.449
<i>S. aureus</i> endocarditis	0.075 0.177	0.145 0.579	0.068 0.153	0.015 0.127	0.101 0.267	0.063 0.217

ELISA readings for peptides 1-6

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Table 8

Indirect ELISA results for Peptide 1

Concentration of peptide: 200µl at 10µg/ml

Primary antibody 1/100

Patient No.	Sera Date	Mean ^a Blank O.D.	Mean Sample O.D. ^b		Standard Deviation	
			IgM	IgG	IgM	IgG
<i>S.mutans</i> Endocarditis						
1	16.03.88	0.131	0.117	0.698	0.001	0.015
2	17.09.87	0.193	0.012	0.742	0.003	0.0017
<i>S.oralis</i> Endocarditis (n=3)						
3	23.05.78	0.141	0.462	0.960	0.018	0.002
4	12.12.81	0.197	0.447	0.902	0.023	0.031
5	23.02.84	0.121	0.043	0.038	0.001	0.008
	30.08.84	0.131	0.153	0.699	0.004	0.007
<i>S.gordonii</i> Endocarditis (n=14)						
6	1.07.86	0.131	0.422	0.936	0.001	0.002
	15.08.86	0.131	0.339	0.855	0.003	0.004
	22.09.86	0.131	0.228	0.81	0.010	0.005
	6.10.96	0.131	0.173	1.06	0.003	0.002
7	8.01.87	0.131	0.487	0.685	0.010	0.012
8	18.12.86	0.131	0.059	0.662	0.004	0.006
	21.01.87	0.131	0.270	0.715	0.005	0.009
9	10.08.86	0.131	0.43	0.41	0.007	0.004
10	17.07.86	0.131	0.568	0.601	0.010	0.012
11	14.07.93	0.131	0.095	0.471	0.012	0.003
12	20.03.93	0.158	0.164	0.404	0.001	0.03
	22.03.93	0.158	0.168	0.475	0.003	0.03
13	06.05.87	0.121	0.438	0.079	0.023	0.003
	12.08.87	0.121	0.274	0.579	0.021	0.016

14	7.06.86	0.131	0.174	0.459	0.006	0.024
	9.06.86	0.151	0.144	0.469	0.004	0.013
	16.06.86	0.153	0.151	0.587	0.005	0.011
	19.06.86	0.193	0.134	0.706	0.006	0.008
	23.06.86	0.151	0.149	0.562	0.007	0.011
	26.06.86	0.153	0.106	0.475	0.005	0.017
15	2.12.87	0.012	0.301	1.391	0.006	0.011
	30.12.87	0.131	0.217	1.083	0.005	0.007
<i>S.sanguis</i> Endocarditis (n=2)						
16	21.07.86	0.121	0.46	0.543	0.006	0.011
	7.08.86	0.121	0.487	0.685	0.001	0.011
17	14.07.93	0.121	0.416	0.401	0.049	0.022
<i>S.oralis</i> Septicaemias (n=8)						
18	23.11.88	0.133	0.046	0.112	0.017	0.001
	6.12.88	0.131	0.024	0.118	0.001	0.004
19	22.08.89	0.131	0.023	0.194	0.001	0.003
	6.09.89	0.144	0.034	0.205	0.0016	0.001
	17.10.89	0.121	0.006	0.042	0.003	0.004
20	11.03.88	0.197	0.007	0.048	0.018	0.008
	1.04.88	0.133	0.043	0.102	0.003	0.011
	10.05.88	0.197	0.021	0.014	0.001	0.001
21	27.06.89	0.197	0.058	0.031	0.015	0.021
	1.80.89	0.151	0.003	0.095	0.002	0.001
22	2.08.88	0.144	0.017	0.082	0.001	0.014
23	4.04.89	0.135	0.044	0.061	0.002	0.002
24	22.03.93	0.193	0.029	0.022	0.001	0.001
	22.04.93	0.158	0.032	0.041	0.003	0.001
	26.04.93	0.139	0.034	0.044	0.011	0.021
	27.04.93	0.158	0.042	0.036	0.001	0.009
25	21.07.93	0.139	0.089	0.027	0.003	0.009
	21.07.93	0.139	0.113	0.031	0.009	0.005
	4.08.93	0.139	0.129	0.032	0.001	0.008
	17.08.93	0.139	0.095	0.103	0.003	0.019
<i>E.faecalis</i> Endocarditis						

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26	25.02.93	0.141	0.027	0.075	0.001	0.014
	25.02.93	0.141	0.024	0.063	0.001	0.003
	29.02.93	0.141	0.026	0.071	0.003	0.001
27	15.06.93	0.147	0.085	0.248	0.003	0.007
	16.06.93	0.147	0.071	0.275	0.002	0.013
28	20.01.92	0.141	0.041	0.069	0.004	0.001
29	24.06.88	0.153	0.085	0.283	0.003	0.017
30	10.06.86	0.135	0.054	0.361	0.003	0.012
31	19.11.87	0.133	0.033	0.652	0.009	0.006
32	29.01.89	0.131	0.081	0.564	0.011	0.016
	13.10.89	0.133	0.024	0.281	0.003	0.022
	16.11.89	0.197	0.016	0.195	0.006	0.005
	8.01.90	0.133	0.043	0.371	0.008	0.018
33	10.05.88	0.197	0.096	1.079	0.003	0.018
	15.05.88	0.131	0.088	1.011	0.003	0.018
	21.05.88	0.131	0.048	0.531	0.002	0.015
	28.08.88	0.131	0.156	0.674	0.003	0.013
34	6.11.86	0.141	0.086	0.082	0.013	0.001
35	12.01.87	0.144	0.039	0.387	0.008	0.022
36	5.01.87	0.135	0.011	0.559	0.001	0.015
<i>E. faecalis</i> Septicaemias						
37	30.12.87	0.133	0.029	0.371	0.001	0.007
38	10.10.85	0.193	0.018	0.175	0.004	0.025
39	24.08.89	0.193	0.033	0.002	0.003	0.002
	11.10.85	0.151	0.052	0.266	0.001	0.008
	11.10.85	0.153	0.049	0.218	0.011	0.014
40	8.01.88	0.135	0.041	0.286	0.019	0.001
41	27.11.87	0.197	0.034	0.354	0.001	0.004
42	20.04.85	0.144	0.02	0.132	0.003	0.006
43	27.06.88	0.144	0.034	0.152	0.013	0.011
<i>E. faecium</i> Septicaemias						
44	19.07.85	0.197	0.135	0.436	0.015	0.026

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45	23.10.85	0.144	0.029	0.269	0.011	0.008
	28.11.85	0.139	0.027	0.061	0.001	0.001
	2.12.85	0.139	0.055	0.057	0.003	0.003
<i>S. bovis</i> Endocarditis						
46	8.07.86	0.133	0.155	0.429	0.023	0.013
47	22.03.85	0.193	0.027	0.021	0.002	0.003
	27.03.85	0.151	0.012	0.153	0.003	0.019
48	12.03.87	0.139	0.255	0.124	0.001	0.007
	6.05.87	0.121	0.438	0.079	0.023	0.003
	12.08.87	0.121	0.274	0.141	0.021	0.016
49	30.10.85	0.131	0.073	0.242	0.011	0.022
50	8.05.86	0.153	0.162	0.593	0.001	0.019
51	12.04.85	0.153	0.203	0.823	0.004	0.015
	26.04.85	0.153	0.241	1.133	0.001	0.001
	30.04.85	0.193	0.025	0.501	0.002	0.011
52	30.12.87	0.144	0.063	0.197	0.003	0.003
53	18.07.93	0.139	0.191	0.069	0.019	0.008
	18.07.93	0.139	0.177	0.066	0.009	0.004
	21.07.93	0.139	0.108	0.035	0.011	0.006
	21.07.93	0.139	0.122	0.038	0.004	0.009
	24.07.93	0.139	0.135	0.054	0.003	0.008
	25.07.93	0.139	0.141	0.073	0.013	0.015
	25.07.93	0.139	0.293	0.051	0.019	0.006
<i>S. agalactiae</i> Endocarditis						
54	20.06.88	0.135	0.025	0.104	0.001	0.005
	22.06.88	0.135	0.088	0.484	0.019	0.011
<i>S. lactis</i> Endocarditis						
55	24.03.88	0.135	0.021	0.218	0.011	0.008
<i>S. pneumoniae</i> Endocarditis						
56	4.12.87	0.133	0.095	0.492	0.011	0.019
57	16.07.86	0.131	0.103	0.281	0.008	0.006
58	7.10.86	0.144	0.101	0.591	0.009	0.003
59	21.10.86	0.131	0.033	0.569	0.011	0.008

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Group G <i>Streptococcal</i> endocarditis						
60	29.10.86	0.131	0.131	0.651	0.009	0.019
61	27.03.85	0.197	0.111	0.789	0.017	0.015
NON NEUTROPENIC CONTROLS						
<i>Staphylococcus aureus</i> Endocarditis						
62	8.02.88	0.149	0.087	0.077	0.029	0.018
63	28.02.88	0.149	0.106	0.037	0.004	0.008
Coagulase Negative <i>Staphylococcus</i> Endocarditis						
64	30.09.89	0.139	0.054	0.062	0.007	0.001
65	16.06.89	0.153	0.051	0.215	0.013	0.007
	1.07.86	0.131	0.146	0.472	0.003	0.015
66	24.02.87	0.139	0.243	0.083	0.001	0.025
	5.03.87	0.121	0.387	0.098	0.008	0.006
67	2.01.88	0.144	0.016	0.192	0.006	0.019
68	10.08.86	0.144	0.029	0.287	0.012	0.019
	25.10.86	0.139	0.067	0.071	0.008	0.006
69	15.02.88	0.133	0.075	0.177	0.011	0.008
Endocarditis due to <i>Candida albicans</i>						
70	24.10.86	0.147	0.244	0.159	0.004	0.003
Endocarditis due to <i>Candida parapsilosis</i>						
71	1.02.88	0.153	0.077	0.192	0.006	0.008
	11.06.88	0.139	0.121	0.165	0.005	0.006
	18.06.88	0.158	0.258	0.216	0.007	0.004
72	8.05.86	0.139	0.087	0.081	0.008	0.007
Endocarditis due to <i>Escherichia coli</i>						
73	1.07.88	0.135	0.038	0.358	0.003	0.018
	2.07.88	0.133	0.022	0.379	0.011	0.011
	5.07.88	0.197	0.073	0.344	0.008	0.002
SLE						
74	13.06.93	0.158	0.327	0.167	0.004	0.011
	16.06.93	0.147	0.322	0.245	0.008	0.022

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75	2.05.88	0.144	0.023	0.285	0.009	0.004
76	18.04.89	0.193	0.015	0.304	0.004	0.003
Brain abscess due to <i>Streptococcus milleri</i>						
77	17.10.86	0.153	0.182	0.312	0.006	0.022
	30.10.86	0.151	0.342	0.353	0.011	0.001
	6.11.86	0.193	0.011	0.185	0.003	0.013
NEUTROPENIC CONTROLS						
78	7.08.88	0.193	0.019	0.183	0.004	0.019
79	11.11.86	0.151	0.101	0.251	0.013	0.009
80	21.10.86	0.149	0.101	0.078	0.015	0.018
81	25.07.91	0.139	0.024	0.045	0.003	0.001
82	9.02.89	0.012	0.151	0.147	0.003	0.003
83	15.06.93	0.151	0.021	0.177	0.004	0.009
84	23.06.92	0.193	0.016	0.176	0.025	0.015
85	19.01.88	0.149	0.108	0.033	0.015	0.001
86	25.10.88	0.193	0.007	0.035	0.005	0.004
87	21.03.86	0.158	0.026	0.045	0.006	0.008
88	18.02.86	0.139	0.037	0.081	0.001	0.008
89	6.08.91	0.144	0.034	0.421	0.005	0.014
90	24.04.87	0.133	0.017	0.023	0.003	0.003
91	12.12.89	0.144	0.013	0.047	0.001	0.004
92	30.10.86	0.139	0.076	0.125	0.009	0.014
93	2.07.86	0.139	0.125	0.094	0.007	0.003
94	5.02.87	0.149	0.119	0.032	0.001	0.003
95	5.09.98	0.135	0.017	0.021	0.004	0.003
96	5.08.93	0.153	0.055	0.152	0.014	0.026
97	19.08.93	0.158	0.022	0.058	0.001	0.004
	1.09.93	0.158	0.044	0.088	0.009	0.006
	14.09.93	0.158	0.026	0.144	0.003	0.022
98	3.05.87	0.141	0.131	0.252	0.004	0.014

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- a* Mean blank O.D., this value was obtained for each microtitre plate by calculation of the mean O.D. for the six wells containing stain only. This mean blank O.D. was subtracted from the each of the sample O.D. measurements to produce the corrected mean sample O.D. value.
- b* Mean sample O.D., this was the value obtained after subtraction of the mean blank O.D. value from the mean of the three O.D. measurements taken from each of the sample wells.

Table 8a

Indirect ELISA results

Concentration of peptides: 200ml at 10ug/ml

Primary antibody 1/100

*Peptide numbers 1,7 and 8 as before.

Patient No	Mean Sample Optical Density					
	IgM			IgG		
	1 ^a	7 ^a	8 ^a	1 ^a	7 ^a	8 ^a
<i>S.mutans</i> Endocarditis						
1	0.117	0.636	0.862	0.698	0.602	0.733
<i>S.oralis</i> Endocarditis						
4	0.447	0.584	1.236	0.902	0.433	0.678
<i>S.gordonii</i> Endocarditis						
6	0.422	0.742	1.443	0.936	0.524	1.694
8	0.27	0.582	0.891	0.715	0.319	1.01
9	0.43	1.103	0.95	0.41	0.388	0.19
10	0.568	0.700	0.824	0.601	0.319	0.505
11	0.095	0.641	1.396	0.471	0.685	0.889
12	0.164	0.988	1.399	0.404	0.828	0.859
13	0.274	1.098	1.605	0.579	0.841	0.799
14	0.174	0.991	1.600	0.459	0.542	0.64
<i>S.sanguis</i> Endocarditis						
16	0.46	1.335	1.366	0.543	1.802	2.267
17	0.417	1.126	1.07	0.401	0.335	0.632
<i>S.oralis</i> Septicaemias						
18	0.046	0.136	0.243	0.112	0.324	0.536
19	0.023	0.324	0.314	0.194	0.294	0.586

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20	0.007	0.228	0.295	0.048	0.272	0.607
21	0.058	0.206	0.280	0.031	0.289	0.476
22	0.017	0.316	0.879	0.982	0.384	0.824
24	0.029	0.911	1.327	0.022	0.475	0.754
25	0.089	0.348	0.720	0.027	0.238	0.369
<i>E. faecalis</i> Endocarditis						
26	0.027	0.344	0.390	0.075	0.376	0.636
32	0.081	0.226	0.317	0.564	0.388	0.756
35	0.039	1.133	1.228	0.387	0.906	1.086
36	0.011	0.549	0.926	0.559	0.591	1.176
<i>E. faecalis</i> Septicaemias						
38	0.018	0.784	1.344	0.175	0.39	0.796
39	0.033	0.590	0.78	0.002	0.432	0.87
<i>E. faecium</i> Septicaemias						
45	0.144	0.47	1.228	0.269	0.588	1.086
<i>S. oralis</i> Endocarditis						
51	0.203	1.063	1.075	0.823	0.410	0.802
<i>S. aureus</i> Endocarditis						
62	0.087	0.333	0.266	0.077	0.435	0.401
63	0.106	0.356	0.805	0.037	0.484	0.646
Coagulase negative Staphylococcus endocarditis						
64	0.054	0.596	0.879	0.062	0.612	0.829
68	0.029	1.502	1.205	0.287	1.446	1.521
SLE						
74	0.327	0.697	1.509	0.167	0.542	1.839

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Brain abscess <i>S.milleri</i>						
77	0.182	1.421	2.162	0.312	0.500	0.781
Neutropenic Controls						
78	0.193	0.570	0.551	0.183	0.804	0.428
79	0.151	0.579	0.491	0.251	0.726	0.428
80	0.149	0.744	0.628	0.078	0.525	0.512
81	0.139	0.422	0.414	0.045	0.530	0.412
82	0.012	0.406	0.382	0.147	0.931	0.809
83	0.151	0.328	0.394	0.177	0.233	0.396
84	0.193	0.601	0.533	0.176	0.950	0.683
85	0.149	0.581	0.34	0.033	0.372	0.60
86	0.193	0.456	0.325	0.035	0.616	0.313
87	0.158	0.610	0.597	0.045	0.542	0.377
88	0.139	0.559	0.475	0.081	0.682	0.497
89	0.144	0.628	0.130	0.421	0.477	0.166
90	0.133	0.142	0.182	0.023	0.280	0.730
91	0.144	0.394	0.305	0.047	0.636	0.317
92	0.139	0.403	0.323	0.125	0.616	0.588
93	0.139	1.021	0.23	0.094	0.858	0.57
94	0.149	0.42	0.47	0.032	0.53	0.432
95	0.135	0.463	0.436	0.021	0.711	0.674
96	0.153	0.568	0.539	0.152	0.760	0.587
97	0.158	0.743	0.650	0.058	0.914	0.535
98	0.141	0.366	0.384	0.252	0.528	0.366

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Table 9

	IgG ≥ 0.6	IgM ≥ 0.4	IgM ≥ 0.4 and IgG ≥ 0.6
<i>S.mutans</i> endocarditis	100%	0%	100%
<i>S.oralis</i> endocarditis	100%	66%	100%
<i>S.gordonii</i> endocarditis	60%	50%	80%
<i>S.sanguis</i> endocarditis	50%	100%	100%
<i>S.oralis</i> septicaemias	0%	0%	0%
<i>E.faecalis</i> endocarditis	18%	0%	18%
<i>E.faecalis</i> septicaemia	0%	0%	0%
<i>E.faecium</i> septicaemia	0%	0%	0%
<i>S.bovis</i> endocarditis	12.5%	12.5%	25%
<i>S.agalactiae</i> endocarditis	0%	0%	0%
<i>S.lactis</i> endocarditis	0%	0%	0%
<i>S.pneumoniae</i> endocarditis	0%	0%	0%
Group G streptococcal endocarditis	100%	0%	100%
<i>Staphylococcus aureus</i> endocarditis	0%	0%	0%
Coagulase Negative endocarditis	0%	0%	0%
<i>Candida albicans</i> endocarditis	0%	0%	0%
<i>Candida parapsilosis</i> endocarditis	0%	0%	0%
<i>E. coli</i> endocarditis	0%	0%	0%
SLE	0%	0%	0%
<i>S.milleri</i>	0%	0%	0%
Neutropenic controls	0%	0%	0%

Table 10

Epitope Number	Epitope Sequence	Negative Control	Viridans Endocarditis	<i>S. oralis</i> Septicaemia	<i>S. mutans</i> Endocarditis	<i>E. faecalis</i> Endocarditis
		n=3	n=8	n=5	n=2	n=2
		O.D. S.D	O.D. S.D.	O.D. S.D.	O.D. S.D.	O.D. S.D.
61 62 63	NFKQGQG	0.400 0.027 0.388 0.034 0.444 0.058	1.039 0.353 0.972 0.347 0.992 0.329	0.429 0.200 0.419 0.230 0.430 0.215	0.833 0.125 0.808 0.122 0.888 0.195	0.588 0.054 0.508 0.027 0.540 0.013
74 75 76 77 78 79	RQPG	0.341 0.019 0.283 0.033 0.282 0.034 0.350 0.045 0.280 0.023 0.282 0.015	0.902 0.319 1.038 0.470 1.053 0.474 0.981 0.358 0.918 0.425 0.781 0.312	0.322 0.211 0.351 0.180 0.455 0.180 0.309 0.070 0.307 0.080 0.271 0.091	0.839 0.004 0.891 0.011 0.917 0.029 1.041 0.043 1.142 0.405 1.005 0.418	0.407 0.120 0.450 0.119 0.493 0.140 0.472 0.130 0.423 0.096 0.373 0.084
90 91 92 93	SWYGAG	0.308 0.054 0.316 0.069 0.344 0.076 0.306 0.007	0.852 0.318 0.924 0.340 0.917 0.429 0.957 0.378	0.287 0.070 0.328 0.080 0.298 0.060 0.444 0.140	1.027 0.192 1.064 0.138 0.914 0.033 1.083 0.225	0.378 0.088 0.428 0.136 0.377 0.115 0.483 0.131
144 145 148 147	GKIRAV	0.306 0.072 0.384 0.071 0.303 0.042 0.395 0.035	0.824 0.318 1.028 0.309 0.852 0.261 0.933 0.285	0.348 0.180 0.437 0.210 0.348 0.150 0.397 0.180	0.779 0.129 0.971 0.199 0.881 0.240 0.944 0.258	0.389 0.115 0.515 0.120 0.438 0.115 0.483 0.131
320 321 322	RLFAQPQ	0.392 0.031 0.453 0.049 0.467 0.124	1.147 0.657 1.209 0.417 1.145 0.356	0.491 0.180 0.548 0.230 0.531 0.190	1.010 0.151 1.284 0.300 1.289 0.346	0.494 0.115 0.649 0.171 0.593 0.116
359 360 361 362 363	AGRPK	0.395 0.043 0.492 0.065 0.407 0.032 0.430 0.008 0.421 0.042	0.921 0.295 1.049 0.374 0.907 0.318 1.054 0.325 0.946 0.279	0.401 0.200 0.428 0.240 0.382 0.190 0.423 0.230 0.391 0.021	0.749 0.177 0.775 0.030 0.657 0.094 0.753 0.091 0.665 0.088	0.480 0.040 0.491 0.062 0.0462 0.067 0.525 0.082 0.477 0.083
376 377 378	PTGYQFD	0.277 0.035 0.415 0.039 0.351 0.062	0.791 0.479 1.045 0.427 0.802 0.290	0.428 0.080 0.359 0.140 0.291 0.140	0.762 0.190 1.548 0.728 1.274 0.793	0.339 0.095 0.487 0.155 0.380 0.151
426 427 428 429 430	YPTVV	0.309 0.081 0.215 0.084 0.325 0.013 0.339 0.046 0.349 0.088	0.852 0.391 0.473 0.161 1.170 0.457 0.964 0.336 0.976 0.328	0.269 0.100 0.225 0.050 0.450 0.180 0.413 0.150 0.465 0.140	1.275 0.617 0.525 0.117 1.143 0.125 1.050 0.179 1.002 0.198	0.410 0.147 0.289 0.030 0.561 0.194 0.488 0.165 0.458 0.139
577 578 579 580 581	LLKKA	0.279 0.042 0.349 0.019 0.351 0.051 0.395 0.077 0.331 0.029	0.863 0.302 0.907 0.260 0.937 0.254 1.035 0.267 0.847 0.261	0.353 0.190 0.410 0.210 0.415 0.210 0.423 0.210 0.581 0.150	0.681 0.142 0.808 0.270 0.882 0.221 0.898 0.183 0.843 0.153	0.466 0.081 0.557 0.103 0.549 0.098 0.587 0.124 0.482 0.141

Table 11

Peptide Number	Viridans Endocarditis	<i>S.oralis</i> Septicaemia	<i>S. mutans</i> Endocarditis	<i>E. faecalis</i> Endocarditis
	n = 8	n = 5	n = 2	n = 2
61	7/8	1/5	2/2	0/2
62	5/8	1/5	2/2	0/2
63	5/8	1/5	2/2	0/2
74	6/8	0/5	2/2	0/2
75	7/8	0/5	2/2	0/2
76	7/8	0/5	2/2	0/2
77	6/8	0/5	2/2	0/2
78	6/8	0/5	2/2	0/2
79	4/8	0/5	2/2	0/2
90	5/8	0/5	2/2	0/2
91	5/8	0/5	2/2	0/2
92	5/8	0/5	2/2	0/2
93	5/8	0/5	2/2	0/2
14	6/8	0/5	1/2	0/2
145	7/8	1/5	2/2	0/2
146	6/8	0/5	2/2	0/2
147	6/8	0/5	2/2	0/2
320	7/8	1/5	2/2	0/2
321	8/8	1/5	2/2	1/2
322	7/8	1/5	2/2	0/2
359	6/8	1/5	1/2	0/2
360	7/8	1/5	2/2	0/2
361	5/8	1/5	1/2	0/2
362	7/8	1/5	1/2	0/2
263	6/8	1/5	1/2	0/2
376	4/8	0/5	1/2	0/2
377	6/8	0/5	2/2	0/2
378	4/8	0/5	2/2	0/2
426	4/8	0/5	2/2	0/2
427	1/8	0/5	0/2	0/2
428	7/8	1/5	2/2	0/2
429	7/8	0/5	2/2	0/2
430	5/8	0/5	2/2	0/2
577	5/8	0/5	1/2	0/2
578	6/8	1/5	1/2	0/2
579	6/8	1/5	2/2	0/2
580	8/8	1/5	2/2	0/2
581	5/8	0/5	2/2	0/2

Positive is > 0.700

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CLAIMS

1. A purified bacterial protein expressed during infection due to streptococci or enterococci and isolated from human sera having at least the sequence of formula (1):

NH-	10	30	50
EFTFYDENDQ	PINFDNALLS	VASLNREHNS	IEMAKDYSGT
			FIKISGSSIG
			EKNGMIYATE
	70	90	110
TLNFKQGQGG	ARWTMYPNRQ	PGSGWDSSDA	PNSWYGAGAI
			SMSGPTNHVT
			VGATSATNVM
	130	150	170
SVAEMPQVPG	RDNTEGKRPN	IWYSLNGKIR	AVDVPKITKE
			KPTPPVAPTE
			PQAPTYEVEK
	190	210	230
PLEPAPVAPS	YENEPTPPVK	TPDQPEPSKP	EEPTYETEKP
			LEPAPVAPNY
			ENEPTPPVKT
	250	270	290
PDQPDPSKPE	EPNYETEKPL	EPAPVAPSYE	NEPTPPVKTP
			DQPEPSKPEE
			PNYDPLPTPP
	310	330	350
LAPTPKQLPT	PPAVPTVHFH	YNRLFAQPQI	NKEIKNEDGV
			DIDRTLVAQO
			SVVKFELKTE
	370	390	410
ALTAGRPKTT	SFVLVDPLPT	GYQFDLEATK	AASKGFETSY
			DKASHTVTFK
			ATEETLAAFN
	430	450	470
ADLTksFETL	YPTVVGRLVN	DGATYTNFT	LTVNDATGVK
			SNIVRVTTTPG
			KPNDDPNPNN
	490	510	530
NYIKPLKVNK	NKQGVNIDGK	EVLASTNYY	ELTWDLDOYK
			GDKSSKEAIQ
			NGFYVDDYP
	550	570	590
EEALTLQPEL	VKIRDLEGNL	VSGISVQQFD	SLERAPKKVQ
			DLLKKANITV
			KGAFQLFSAD
	610		
NPAEF			

or an immunogenic fragment thereof or an analogue thereof.

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2. A bacterial protein according to claim 1 further characterised by either one or both of the following features:-

(1) It is an immunodominant conserved antigen; and

(2) Recombinant human antibody in an animal model (mouse) protected against septicaemia infection;

3. A bacterial protein according to either one of claims 1 or 2 further characterised in that it is involved in binding to heart valves.

4. A bacterial protein or fragment or analogue according to any one of claims 1 to 3 wherein the protein is obtained from any one of the group of Streptococcus oralis, Streptococcus sobrinus, Streptococcus gordonii, Streptococcus sanguis, Streptococcus mutans, Streptococcus mitis, Streptococcus mitior, Streptococcus parasanguis, Streptococcus bovis, Enterococcus faecalis and Enterococcus faecium.

5. A bacterial protein or fragment or analogue according to any one of claims 1 to 3 wherein the protein is obtained from either one of the group of vancomycin-resistant Enterococcus faecalis and Enterococcus faecium.

6. A bacterial protein or fragment or analogue according to any one of claims 1 -5 wherein it is a recombinant protein, fragment or analogue.

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7. A fragment or an analogue thereof according to any one of claims 1 - 6 wherein it comprises any one of the sequences of YEVEKPLEPAPVAPS, SYENEPTPPVKTPD, KTPDQPEPSKPEEPT, EPAPVAPSYENEPTP, YEVEKELVDLPVEPS, KTPDQNIPDKPVEPT, TMYPNRQPGSGWDSS and WYSLNGKIRAVDVPK.
8. A derivative of a protein, fragment or analogue according to any one of claims 1 to 7 wherein the derivative inhibits said protein.
9. An inhibitor according to claim 8 wherein it comprises isolated and purified antibody specific to the protein, fragment or analogue of any one of the preceding claims.
10. An antibody according to claim 9 wherein it is either monoclonal or polyclonal.
11. An antibody according to either one of claims 9 or 10 wherein it comprises a whole antibody or an antigen binding fragment thereof.
12. An antigen binding fragment according to claim 11 wherein it comprises any one of the group of F(ab')₂, Fab', Fab and Fv fragments.
13. An antibody or antigen binding fragment according to any one of claims 9 to 12 wherein it is a human recombinant antibody or antigen binding fragment.

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14. The protein, fragment, analogue, inhibitor, antibody or antigen binding fragment according to any one of the preceding claims when used in a method of diagnosis or treatment of the human or animal body.

15. The protein, fragment, analogue, inhibitor, antibody or antigen binding fragment according to claim 14 wherein the diagnostic test method is selected from one of the group of enzyme-linked immunosorbent assay, radioimmunoassay, latex agglutination assay and immunoblot assay.

16. A composition for use in a method of diagnosis or treatment of the human or animal body comprising the protein, fragment, analogue, inhibitor, antibody or antigen binding fragment of any one of claims 1 to 13 together with a pharmaceutically acceptable carrier, diluent or excipient.

17. A DNA sequence coding for a bacterial protein or an immunogenic fragment or an analogue thereof expressed during infection due to streptococci or enterococci having substantially the nucleotide sequence of formula (2):

```

5'      10      30      50
GAATTCACCT TCTACGATGA AAATGACCAA CCAATTAATT TTGACAATGC TCTTCTTTCA
      70      90      110
GTAGCCTCAC TTAACCGTGA GCATAACTCT ATTGAGATGG CTAAGGATTA TAGTGGTACT
      130     150     170
TTTATTAAAA TCTCAGGTTC ATCCATCGGT GAAAAAATG GCATGATTTA TGCCACAGAA
      190     210     230
ACCCTGAACT TAAACAAGG ACAGGGTGGG GCTCGCTGGA CAATGTATCC AAATCGTCAG
      250     270     290

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CCAGGTTTCAG GTTGGGATTC ATCAGATGCA CCAAACCTCTT GGTACGGTGC AGGGGCCATT

310	330	350
AGTATGTCCG	GTCCTACGAA	TCACGTTACA
370	390	410
TCCGTAGCAG	AAATGCCTCA	AGTACCTGGA
430	450	470
ATCTGGTACT	CACTCAATGG	TAAAATTCGT
490	510	530
AAACCAACTC	CACCGGTAGC	ACCAACTGAA
550	570	590
CCACTGGAAC	CGGCTCCAGT	AGCACCAAGC
610	630	650
ACTCCAGATC	AACCGGAGCC	ATCAAAACCA
670	690	710
TTGGAACCAG	CTCCAGTAGC	ACCAAACTAC
730	750	770
CCAGATCAAC	CAGACCCATC	AAAACCGGAA
790	810	830
GAACCAGCTC	CAGTAGCACC	AAGCTATGAA
850	870	890
GATCAACCAG	AGCCATCAAA	ACCAGAAGAG
910	930	950
CTAGCACCAA	CTCCTAAGCA	GTTGCCAACA
970	990	1010
TACAATCGTC	TATTTGCACA	ACCTCAGATT
1030	1050	1070
GATATTGATC	GTA CTCTAGT	TGCTAAGCAG
1090	1110	1130
GCTTTAACTG	CTGGTCGTCC	AAAAACA ACT
	TCGTTTGTAT	TGGTAGATCC
		ACTTCCA ACT

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1150	1170	1190
GGCTATCAGT	TTGATTTGGA	AGCAACCAAG
1210	1230	1250
GACAAAGCTA	GTCACACTGT	AACCTTTAAG
1270	1290	1310
GCTGATTTGA	CAAAATCCTT	TGAGACTCTA
1330	1350	1370
GATGGGGCGA	CTTATACGAA	TAACCTTTACA
1390	1410	1430
TCAAACATTG	TTCGTGTAAC	GACTCCAGGT
1450	1470	1490
AACTACATCA	AGCCTTTGAA	AGTTAACAAG
1510	1530	1550
GAAGTTCTAG	CTGGTTCAAC	GAAGTTCTAG
1570	1590	1610
GGAGATAAAT	CTTCTAAAGA	AGCGATTCAA
1630	1650	1670
GAAGAAGCTT	TAACGCTTCA	ACCAGAATTG
1690	1710	1730
GTATCAGGTA	TCAGTGTTCA	ACAGTTTGAT
1750	1770	1790
GATCTGTTGA	AGAAAGCAAA	CATCACTGTT
1810		
AATCCAGCTG	AATTC	

and homologues thereof.

18. A DNA sequence according to claim 17, the protein for which it encodes being further characterised by either one or both of the following features:-

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- (1) It is an immunodominant conserved antigen; and
 - (2) Recombinant human antibody in an animal model (mouse) protected against septicaemia infection; and
19. A DNA sequence according to either one of claims 17 or 18 further characterised in that the protein for which it encodes is involved in binding to heart valves.
20. An expression vector including substantially the DNA sequence of any one of claims 17 to 19.
21. A DNA probe specific to the DNA sequence of any one of claims 17 to 19.
22. A derivative of a DNA sequence according to any one of claims 17 to 19 wherein it inhibits the synthesis or activity of the protein.
23. The DNA sequence, vector, probe or inhibitor according to any one of claims 17 to 22 when used in a method of treatment or diagnosis of the human or animal body.
24. Fibronectin or an immunogenic fragment thereof or an analogue thereof or an antibody thereto or an antigen binding fragment thereof when used in a method of treatment or diagnosis of the human or animal body for infection due to Streptococci or Enterococci.

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25. Fibronectin or an immunogenic fragment thereof according to claim 24 wherein the fibronectin is human fibronectin.

26. Antibodies specific to HSP 90 or immunogenic fragments or analogues thereof for use in a method of diagnosis or treatment of the human or animal body of infection due to streptococci or enterococci due to any one of the group of S.oralis, S.gordonii and S.sanguis.

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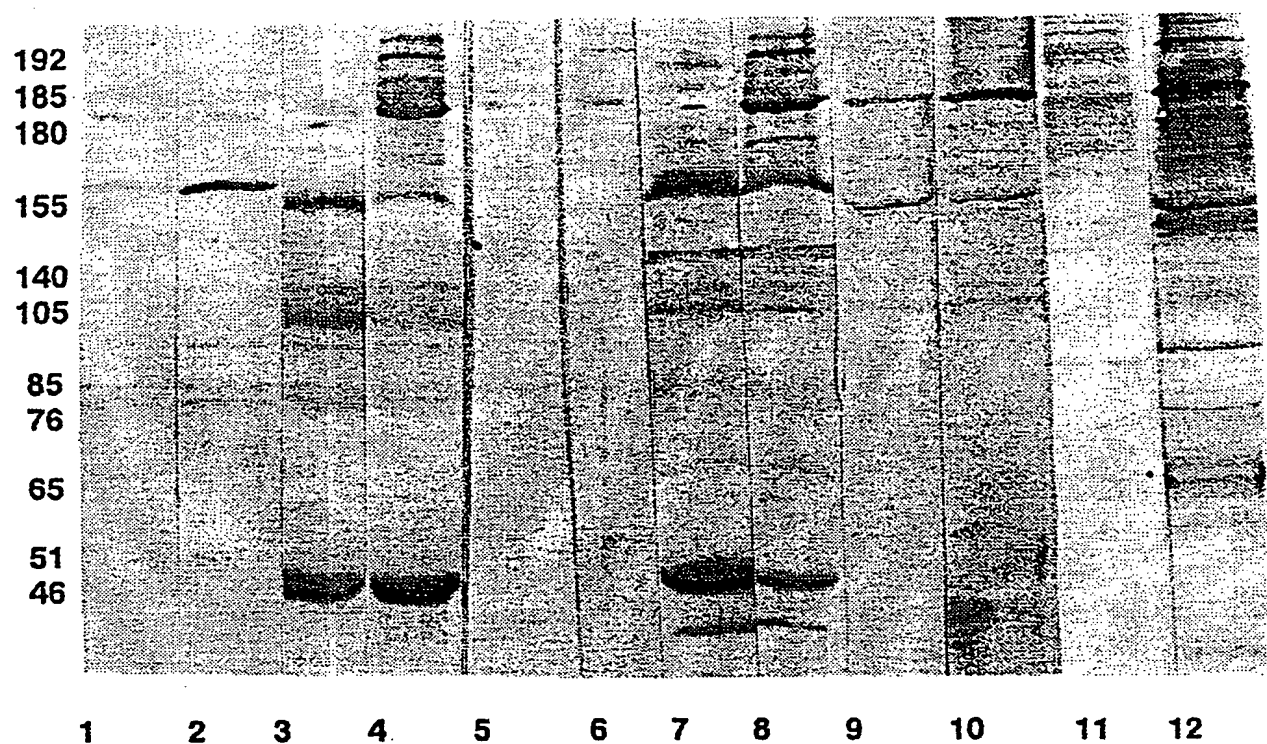


FIGURE 1

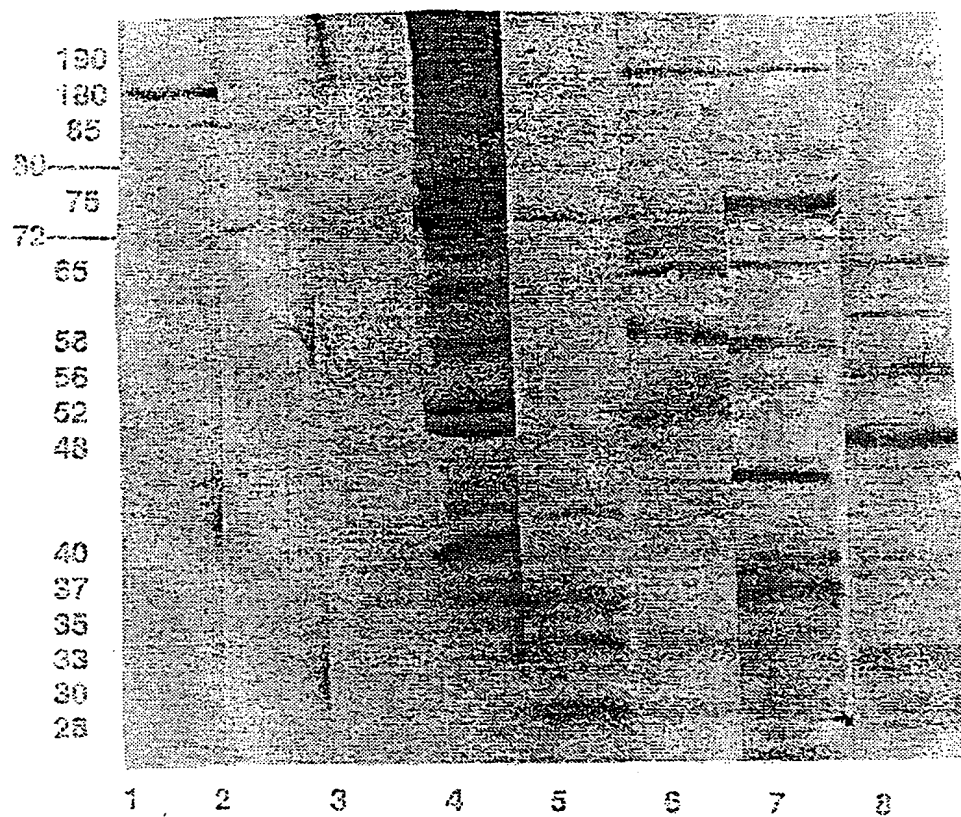
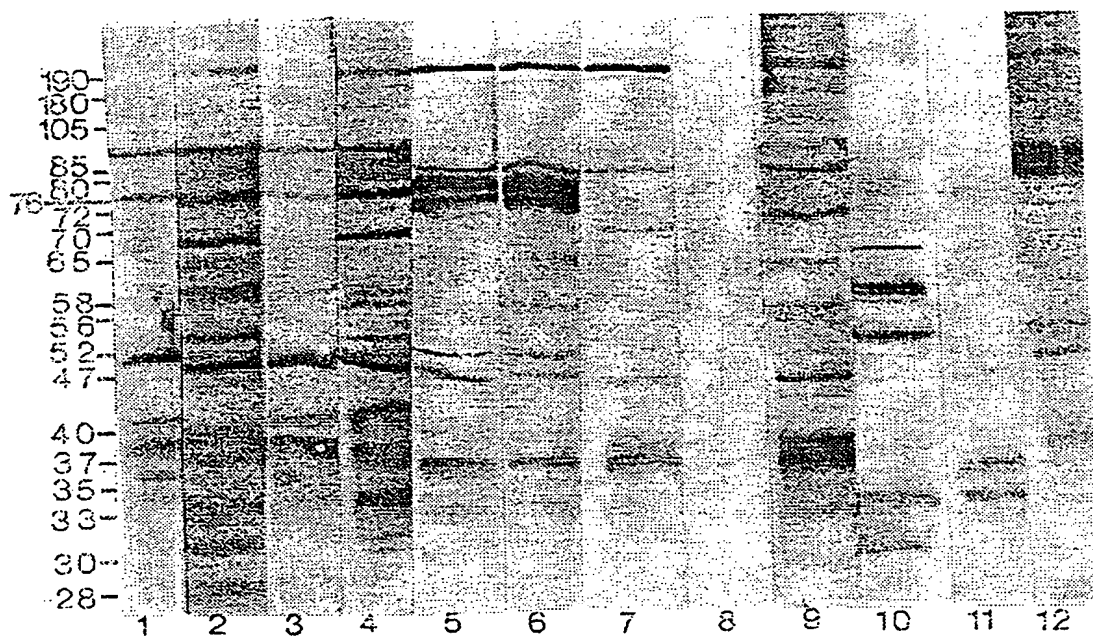


FIGURE 2

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*Figure 3*

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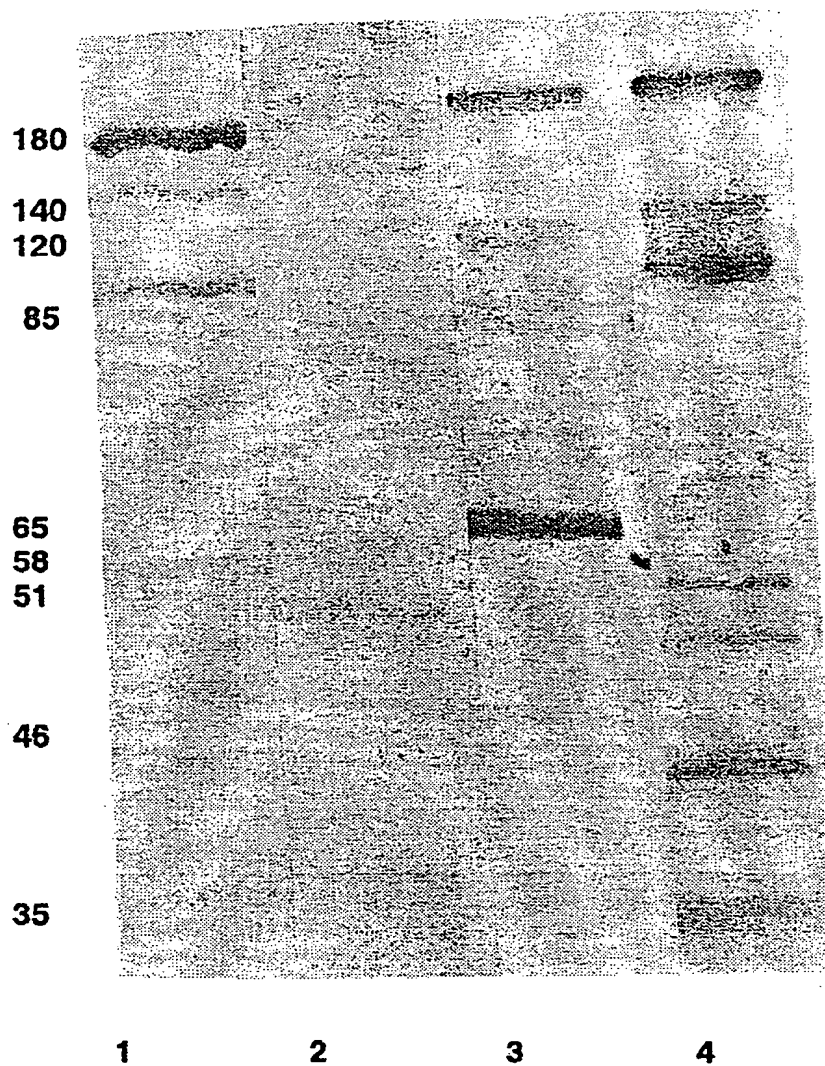


FIGURE 4

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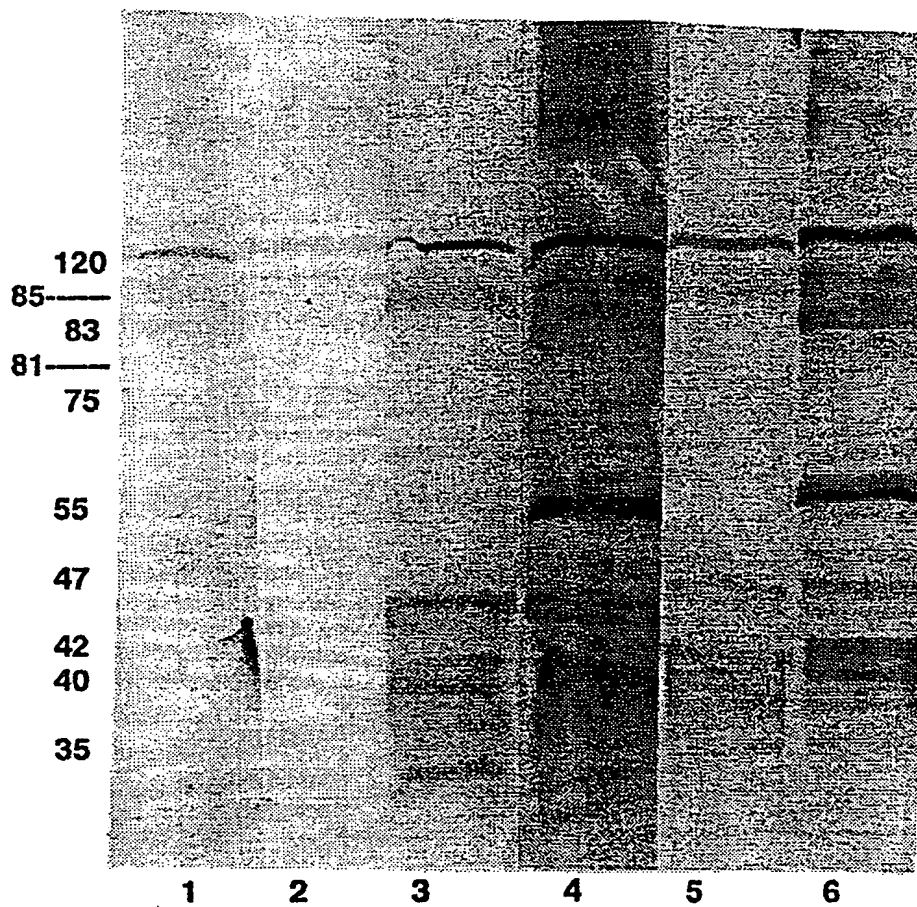


FIGURE 5

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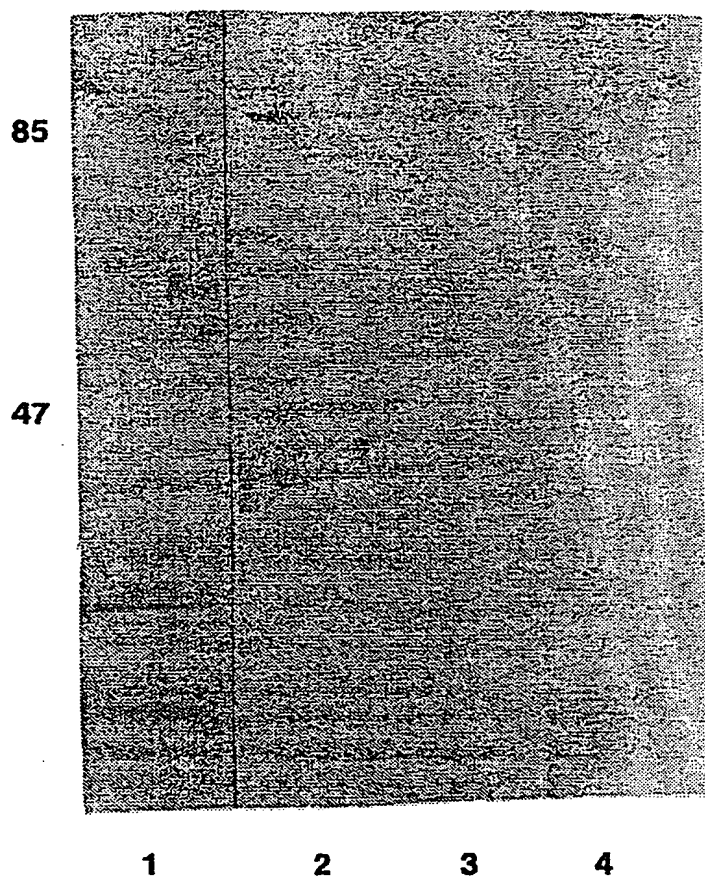


FIGURE 6

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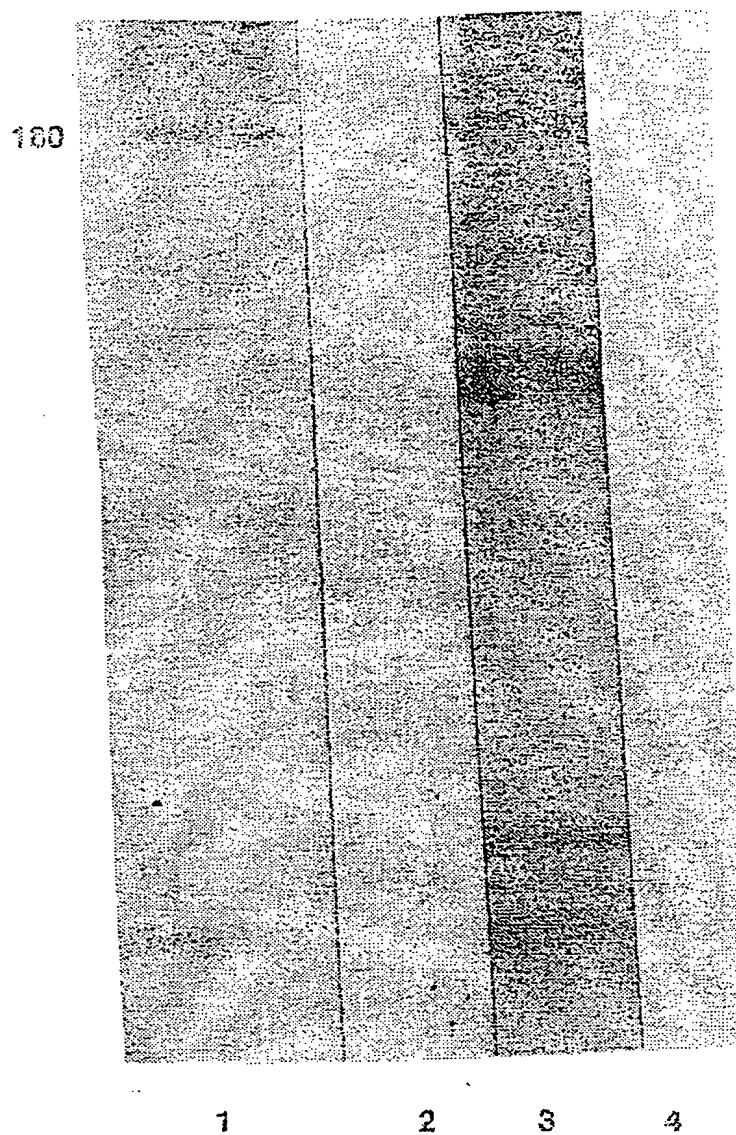
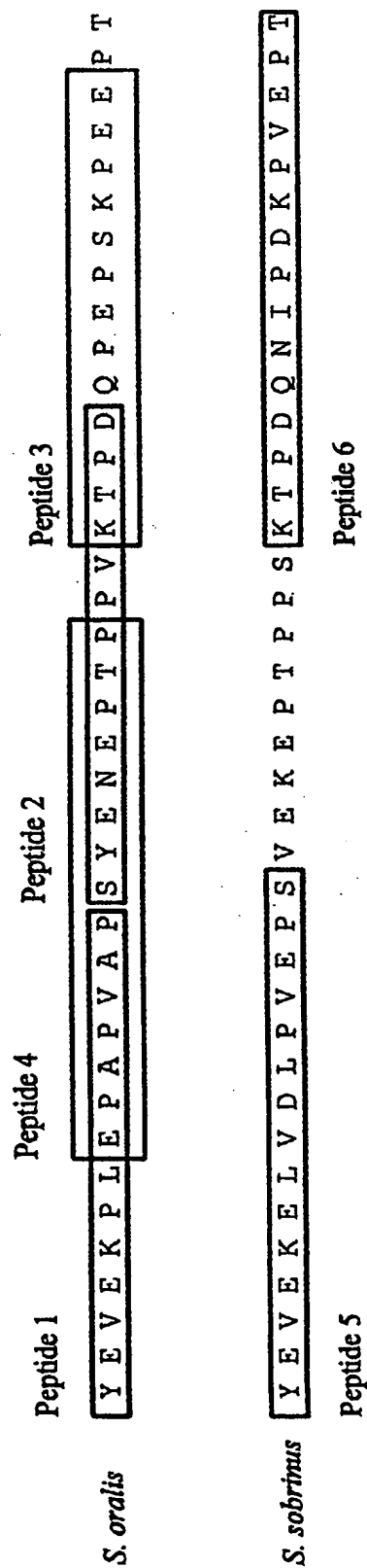


FIGURE 7

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Figure 8



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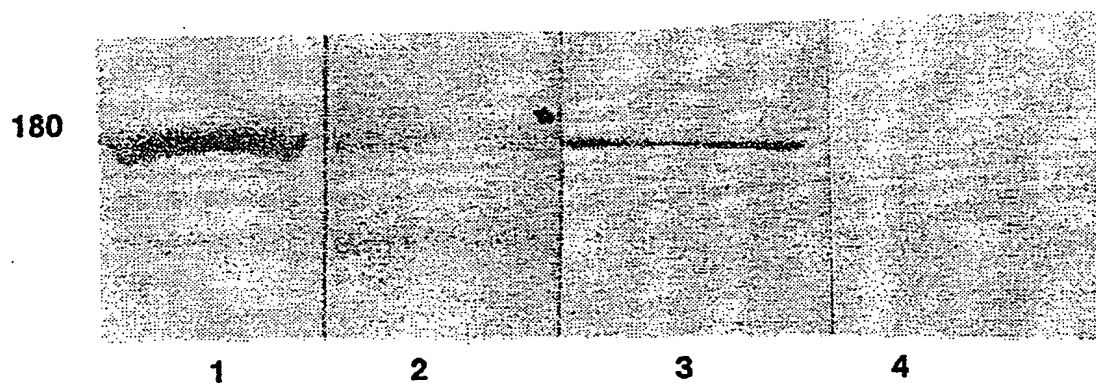


FIGURE 9

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Figure 10

Gap alignment of the *S. sobrinus* antigenic region
with the human Fibronectin primary sequence

	710	730	750
FN HUM	STSTPVTSNT VTGETIPFSP LVATSESVTE ITASSFVVS	VSASDTVSGF	
ESA STRSOY
	760	770	790
FN HUM	RVEYELSEEG DEPOYLDLPS TATSVNIPDL LPGRKYIVNV	YQISEDGEQS	
ESA STRSO	EVEKELVDLP VEPSYEKEPT PPS.....	
	810	830	850
FN HUM	LILSTSQTTA PDAPPDPTVD QVDDTSIVVR WSRPQATITG	YRIVYSPSVE	
ESA STRSO	...KTPDQNI PDKPVEPT..	

Key for Figure 10:

FN HUM the human fibronectin primary sequence homologous to the ESA
 STRSO peptin region.

ESA STRSO the proposed *S. sobrinus* endocarditis specific antigenic site.

The numbers correspond to the amino acids of the complete human fibronectin
 primary sequence.

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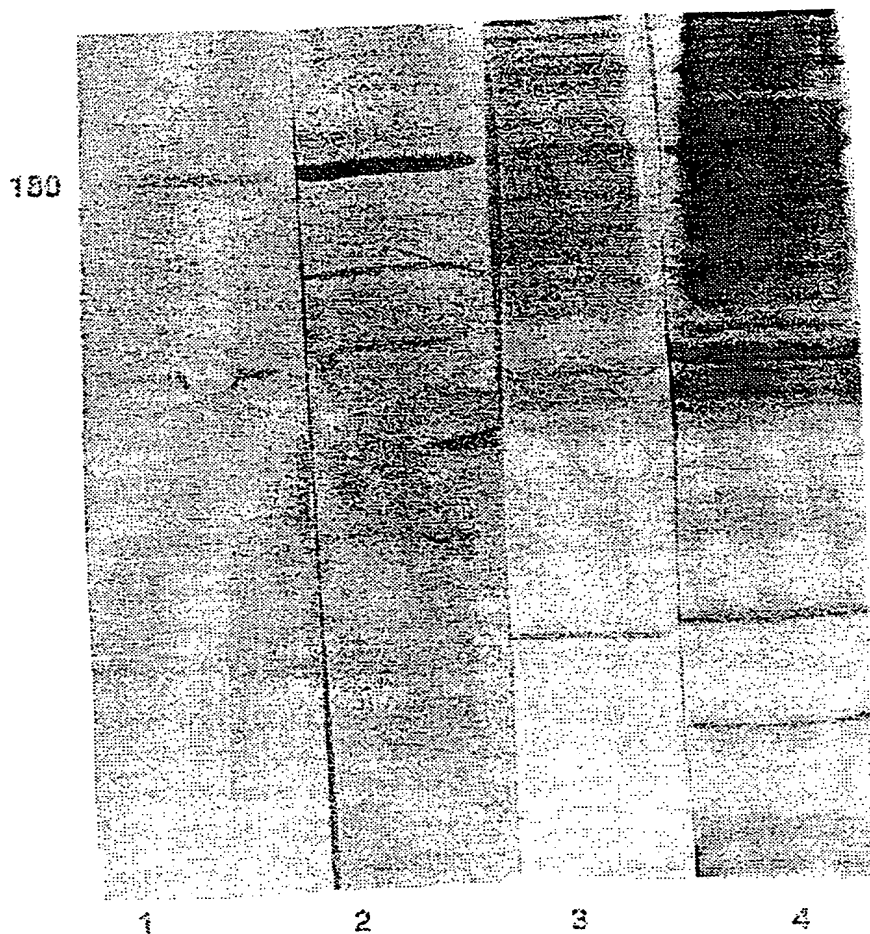


FIGURE 11